

for those cancers are less consistent but should not be dismissed. The draft IRIS assessment considered those end points but did not weigh them heavily in the classification of tetrachloroethylene as a human carcinogen. That is appropriate.

GENERAL COMMENTS ON THE ENVIRONMENTAL PROTECTION AGENCY'S PRESENTATION OF EPIDEMIOLOGIC EVIDENCE ON CANCER

One of the biggest difficulties in assessing the cogency of the EPA's assessment related to cancer is how the data are organized in the tables and some parts of the text. It would be much easier to evaluate the overall picture of results regarding tetrachloroethylene and a particular cancer if the tables were organized by cancer type as opposed to the current format, which organizes them by study design. The current format requires the reader to jump between sections for cohort mortality, incidence, and case-control studies. Studies are sometimes further categorized as to the type of worker included (for example, dry-cleaner vs degreaser); this makes it extremely difficult to evaluate the overall consistency or lack of consistency in results related to specific cancers.

Errors in reporting results also occur occasionally. For example, the draft reports (on page 4-150, lines 1-3), in relation to Hodgkin disease, "a statistically significantly elevated risk for male [sic] with a job title of dry cleaner or laundry worker (Costantini et al. 2001)." The result from Costantini et al. for that group in relation to Hodgkin disease was an OR of 2.5 (95% CI, 0.3-24.6), which is not significant and was based on a single case.

The overall impression is that data are presented to support a positive association between tetrachloroethylene and cancer and that studies that found no such association are criticized or minimized. EPA should provide a clearer discussion of criteria used to identify studies of merit and a more balanced critique to strengthen the draft IRIS assessment.

RESEARCH RECOMMENDATIONS

Population-based studies, preferably in well-defined occupational cohorts, that can measure both cancer incidence and mortality and have sophisticated exposure reconstruction components that are specific to tetrachloroethylene would add significantly to the literature. The studies must also be adequately controlled for the effects of smoking and alcohol consumption to address the lingering questions of the association between tetrachloroethylene and esophageal cancer. In the absence of data to control for these confounders, sensitivity analyses should be conducted to estimate the exposure effect after adjustment under reasonable sets of assumptions regarding smoking prevalence and the strength of smoking effects. Further research that classifies exposure only by occupational title will not add to the literature.

Reference Values for Tetrachloroethylene

The Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of tetrachloroethylene provides the agency's assessment of the potential human health effects of exposure to the chemical. For noncancer effects, EPA proposes to establish an oral reference dose (RfD) and an inhalation reference concentration (RfC), which the agency defines as estimates (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure and a continuous inhalation exposure, respectively, of the human population (including sensitive subgroups) that are likely not to pose an appreciable risk of deleterious effects during a lifetime. The proposed RfC for tetrachloroethylene is 0.016 mg/m³, and the proposed RfD is 0.004 mg/kg per day. This chapter discusses how those reference values were determined.

SELECTION OF CRITICAL END POINT AND STUDIES

EPA selected neurotoxicity—specifically, outcomes of visual and cognitive dysfunction—as the critical noncancer health effect of tetrachloroethylene. As described in Chapter 3, epidemiologic and human studies involving controlled exposures have provided evidence of those effects. The experimental-animal literature available when the draft IRIS document was written also provided strong evidence that tetrachloroethylene is neurotoxic. One study (Mattsson et al. 1998) offered support of effects on visual function. New studies have provided further support of effects on the visual system and signal-detection tasks (Oshiro et al. 2008; Boyes et al. 2009) in animals. Although the committee supports EPA's decision to use neurotoxicity as a critical end point, it recommends more focused assessments of specific criteria related to study design and methods as part of the process of selecting critical studies for deriving reference values.

The committee found that EPA reviewed all the relevant studies available at the time that the draft was written and agrees with many of the limitations that are noted, beginning on page 4-101. The committee also found, however, that the draft sometimes failed to consider weaknesses in study methods or inconsis-

tencies in results, two factors that should carry great weight in selecting key studies for calculating an RfC. For example, test outcomes (neurologic signs, emotional lability, choice reaction time, cancellation d2, and digit symbol) in a study by Seeber (1989) were worse in the low-exposure group compared with the high-exposure group. EPA's discussion of the study (Section 4.6.1.2.2) did not mention that discrepancy. In another example, the committee judged the study by Echeverria et al. (1995) to be stronger than is characterized in the draft assessment (see detailed discussion in Chapter 3 of the present report). EPA discounted the study because (p. 4-77 to 4-78) "the lack of an unexposed control group limits the ability of the study to fully characterize the magnitude of the effects on visuospatial ability and to detect exposure-related symptoms or effects on tests of non-visuospatial cognitive ability. It also limits the extrapolation of the results to other populations exposed to tetrachloroethylene." The committee judged that although there was no unexposed comparison group, the use of an internal comparison group (the group with the lowest exposure) has the advantage that any selection and confounding factors related to working in dry-cleaning facilities are present in both groups and reduces potential confounding by unmeasured factors.

The committee applied several criteria in selecting the epidemiologic studies that it considered most useful in establishing reference values for tetrachloroethylene. Three general criteria were addressed: the validity of individual studies, the internal consistency of results with the hypothesis of a causal role for tetrachloroethylene (for example, is there an association in a low-exposure group but not in the high-exposure group?), and the consistency of the findings with what is known from other sources (how the study fits into the overall picture of what is known). Those criteria are discussed in detail in Chapter 3.

EPA selected the study by Altmann et al. (1995), conducted in Mülheim, Germany, for calculating the RfC because it involved environmental exposures that are more relevant than occupational exposures for determining values designed to protect public health and it used a standardized computer-assisted testing battery. Those study factors are reasonable considerations, but they are not the most relevant for selecting a critical study. The committee concluded that the validity of the 1995 Altmann et al. study was seriously compromised by methodologic deficiencies, which are discussed in detail in Chapter 3 and summarized briefly below.

- The most important concern is that the referent group was inappropriate in that it did not represent the counterfactual example. It was selected from among employees of the Public Health Office or the Medical Institution of Environmental Hygiene in Mülheim and matched to exposed subjects by age and sex. This selection bias resulted in a reference group clearly was more educated than the exposed group, and because the authors used only three categories of education, it is unlikely that differences in education were adequately controlled for. Because several of the primary outcomes are influenced by education, it is likely that substantial confounding remained. For example, there was no association between

tetrachloroethylene and visual evoked potentials (VEPs). That is important because visual deficits have been the most consistently reported effects of tetrachloroethylene, and they are outcomes that are essentially unrelated to education. Measures of vigilance, attention, and visual memory are strongly associated with education, and the exposed group had poorer performance in them, whereas measures of eye-hand coordination and finger tapping, which are weakly related to education, were similar in the two groups.

- The rationale for the selection of study participants was poorly described, and several of the exposure measurements in those supposedly exposed were not reported. Without that information, it is impossible to determine whether this was a biased sample (that is, whether others were excluded for reasons other than study design).

- Tetrachloroethylene was measured in air samples from homes for 7 days. Figure 1B of the paper is supposed to show indoor air concentrations for exposed participants and referents, but no concentrations are shown for the referent group. The amount of time that residents spent in their apartments is unknown. Time out of the apartments before neurobehavioral testing was unknown but was believed to account for lower blood concentrations of tetrachloroethylene before testing.

- In the analyses, exposure was defined by group membership (yes-no variable) rather than by markers of exposure. Therefore, no dose-effect relationship was established in the exposed group. As stated above, group differences in neurobehavioral performance were more likely to be related to residual confounding by education or pre-exposure intellectual capacity.

- The Neurobehavioral Evaluation System battery used to assess brain dysfunction related to exposure appropriately included four subtests that have been shown to be associated with solvent exposure in other research. However, the battery has no norms in this population, and some of the tests have not been well validated with regard to what they reveal about brain damage from any cause. The absence of norms makes it especially important to have basic, standardized measures of intellectual function that can be used to characterize the longstanding cognitive abilities (native intellectual capacity) of the two groups so that differences between the groups can be correctly attributed to exposure.

On the basis of the study selection criteria noted earlier—which emphasized validity, methodology, and consistency with the literature—the human studies that the committee judged most appropriate to use as points of departure for derivation of the RfC are Altmann et al. (1990), Cavalleri et al. (1994), Gobba et al. (1998), and Echeverria et al. (1995). The details of those studies and the reasons for their selection are discussed fully in Chapter 3 and summarized briefly here. The study by Altmann et al. (1990), who used controlled exposures in an experimental chamber, was chosen because it used random assignment to exposure groups, which reduced the potential for confounding of any associations between exposure and outcomes, and the exposure dosage was known. The study by Cavalleri et al.

(1994) was useful because it examined an occupational cohort of 33 dry-cleaners and it included followup assessments 2 years later, as reported by Gobba et al. (1998). Some members of the cohort continued to be exposed to the same workplace concentrations of tetrachloroethylene, and others worked in facilities where exposures had been reduced. The 1998 study by Gobba et al. was useful in that it allowed assessment of color vision before and after alterations in workplace exposure to tetrachloroethylene and because exposure concentrations could be estimated. The primary advantages of the study by Echeverria et al. (1995) were the reduction in potential confounding and confounding due to the use of an internal referent group and the ability to examine exposed workers for a dose-response effect with respect to measures of visuospatial performance on the basis of estimated cumulative lifetime exposure to tetrachloroethylene.

Among the animal studies considered by the committee, the one by Boyes et al. (2009) was judged to be appropriate to use as a point of departure for derivation of the RfC. The most sensitive end point in the study was the F2 (frequency-doubling) component of the evoked potential spectrum, a measure thought to reflect the activity of cortical neurons that respond to both stimulus offset and onset. The investigators also conducted a toxicokinetic analysis relating exposure concentration and duration to brain concentration. From that analysis, brain concentrations of tetrachloroethylene were linked to visual function.

DOSE METRICS

With respect to neurotoxicity, EPA's use of the blood area under the curve (AUC) for tetrachloroethylene with various routes of exposure appears to be justified. The physiologically based pharmacokinetic (PBPK) model simulations presented in Figures 3-4, 3-5, 3-6a, and 3-6b of the draft IRIS assessment (EPA 2008) do suggest that the three PBPK models collectively describe the variation in blood and exhaled-breath concentrations of tetrachloroethylene observed in controlled human exposures. That provides confidence that later calculations of the tetrachloroethylene AUC during various exposure scenarios are accurately captured. A better dose metric for use in the neurotoxicity assessment might be the AUC for tetrachloroethylene in the brain. However, given the rapid partitioning of tetrachloroethylene between blood and well-perfused tissues and the lack of experimental data on brain tetrachloroethylene concentrations, the use of the blood AUC as a surrogate was appropriate. (As noted in Chapter 3, there are now some data that might be used in developing PBPK models of brain concentration.)

ROUTE-TO-ROUTE EXTRAPOLATION

EPA has chosen to use the venous-blood AUC as the route-to-route dose metric for extrapolating an inhalation exposure of tetrachloroethylene to a corresponding oral equivalent dose. The rationale behind that approach is sound and

adequately explained in the draft. However, the implementation of the approach raises serious methodologic concerns related to inappropriate use of the selected PBPK models and uncertainties in the fraction of an oral tetrachloroethylene dose that is metabolized. All three of the selected PBPK models were formulated and validated specifically against inhalation exposures. There was no attempt to validate model predictions against blood tetrachloroethylene concentrations after oral dosing. To use the PBPK models, EPA has empirically assumed a value for the rate of oral absorption of tetrachloroethylene, which is entered as a constant in the models. That approach is inferior to direct estimation as was used in other published PBPK models, such as the Gearhart et al. (1993) or Dallas et al. (1995) models (the latter only for rats and dogs). The latter PBPK models might have been better choices to begin this exercise. Better still, a harmonized PBPK modeling approach to synthesize important aspects of the various models into a single model would have provided the greatest confidence in the route-to-route extrapolation. See Chapter 11 for further discussion of the limitations of the PBPK modeling and the proposal to develop a harmonized model.

CHARACTERIZATION OF UNCERTAINTIES

The committee reviewed EPA's application of uncertainty factors in deriving sample reference values on the basis of different studies. It found that the narrative made it clear what uncertainty factors were used but that there were some instances in which a supporting rationale was not provided for departure from the default option and other instances in which departures from the default option should have been considered.

Extrapolation from Lowest Observed-Adverse-Effect Level to No-Observed-Adverse-Effect Level

A factor of 10 was used consistently by EPA when a lowest observed-adverse-effect level (LOAEL) from a study was used instead of a no-observed-adverse-effect level (NOAEL). That is consistent with EPA policy. A benchmark dose (BMD) can be treated as a NOAEL, but no studies of neurotoxicity that could support a BMD calculation had been published when the draft was written. More recent studies of neurotoxicity would support such a calculation (Oshiro et al. 2008; Benignus et al. 2009; Boyes et al. 2009).

Extrapolation from Animals to Humans

The uncertainty factor for extrapolating animal data to humans is considered to have toxicokinetic and toxicodynamic aspects. EPA judged that an uncertainty factor of 3 was adequate to address these uncertainties. EPA applied that approach consistently, but the rationale for doing so was not adequately

described. Specifically, the draft cites an EPA (1994) document, but it would have enhanced transparency if it summarized briefly why an uncertainty factor of 3, rather than the default factor of 10, was used.

Human Variation

The application of a default factor of 10 to account for interindividual variation is justified because of the paucity of data on sensitive populations, including developing and aging organisms. Its use is appropriate and in accordance with EPA guidance.

Extrapolation from Subchronic Exposure to Chronic Exposure

The criteria for selecting the value of the uncertainty factor for extrapolating from subchronic exposure to chronic exposure were not clear, and this uncertainty was handled inconsistently in the draft IRIS assessment. It was noted (p. 5-13) that “a factor to address the potential for more severe toxicity from chronic or lifetime exposure to tetrachloroethylene is not used in this assessment. The epidemiologic studies, except for Schreiber et al. (2002), are all of median duration of exposures of more than 15% of a 70-year lifespan. There are no data to suggest that continuing exposure to tetrachloroethylene can increase the severity of effects; duration-response trends are not generally evident in the human studies.” On the basis of that rationale, no uncertainty factor for extrapolating to lifetime exposure was applied to the Altmann et al. (1995) study. However, in the discussion of the studies that support the RfC, a factor of 10 was applied to the Schreiber et al. study of day-care workers even though the mean exposure period was said to be 4 years, during which 23% of the time would involve exposure. It is not clear how that pattern would differ from residential exposure of people who work outside the home during the day. More directly, however, if EPA believes that longer exposures do not increase neurotoxicity or, by implication, that shorter exposures do not diminish it, one may question why a factor of 10 is applied to the results of the Schreiber et al. (2002) occupational study but not to the results of other occupational studies. Overall, the committee found that the literature provides little information about the possibility of cumulative toxicity from chronic exposure to tetrachloroethylene. No animal studies of chronic, life-long exposure were located, and except for Gobba et al. (1998) the epidemiologic studies did not involve long-term followup.

There is inconsistent use of the uncertainty factor when the sample reference value is based on the results of animal studies—Mattsson et al. (1998) and Rosengren et al. (1986). A factor of 10 was applied to the Mattsson et al. results and a factor of 3 to the Rosengren et al. results even though the two studies were of similar duration. EPA’s rationale (p. 5-15) was that “a subchronic to chronic factor of 3, rather than 10, was applied for Rosengren et al. (1986) in light of the large overall uncertainty for this study associated with extrapolating from a

LOAEL to NOAEL, from animal to humans, for human variation, and for database deficiencies; the total uncertainty factor was 3,000.” That justification is not clear. The reason for modifying the uncertainty factor may be that it is EPA’s *policy* to limit the overall uncertainty to 3,000 in deriving RfCs (EPA 2002). If that is the reason, it should be stated explicitly. If not, better justification should be provided.

The committee believes that an uncertainty factor of 3 should have been considered for animal studies like that of Mattsson et al. (1998) in which exposure occurred for 6 hours/day 4 days/week for 13 weeks. If that exposure regimen is treated in the same manner as acute exposure by applying a higher factor, doing so should be justified. Some discussion of the issue would improve the draft IRIS assessment.

Database Deficiencies

In the derivation of RfCs on the basis of neurotoxicity, EPA used a factor of 3 for database deficiencies because of the inadequacy of the experimental literature designed to characterize hazard and dose-response. Key deficiencies identified were inadequate data to address childhood or other life-stage susceptibility, a paucity of animal studies (especially studies of developing animals and of chronic, low-level exposures) designed to investigate neurotoxicity or to define and characterize dose-response relationships, and inadequate database on cognitive testing. It was unclear whether a factor of 3 was adequate to address these uncertainties because there was some overlap with the factor of 10 applied for human variation, which also addressed developmental concerns.

The committee recommends that EPA revisit and defend more clearly its decision to apply a factor of 3 for database deficiencies in light of new data and the committee’s findings in Chapter 3. New studies include, for example, recent papers from researchers in EPA’s National Health and Environmental Effects Research Laboratory provide excellent data from well-designed studies using controlled, acute exposures that link deficits in visual function and signal detection with atmospheric tetrachloroethylene concentrations and instantaneous concentrations in the brain. This includes papers by Oshiro et al. (2008) and Boyes et al. (2009) investigating function and by Shafer et al. (2005) on mechanisms, which is described in the IRIS document but not fully integrated. These studies link neural or behavioral effects to actual brain concentrations of tetrachloroethylene or to their estimated concentration using PBPK modeling. Thus, the animal literature on controlled acute exposure is now stronger. Notable gaps in the animal literature still include the paucity of studies of developmental or chronic exposures. Another consideration is that the committee found the human study of exposed children (Schreiber et al. 2002) to be methodologically flawed. The committee judges these to be serious gaps in the database, which suggests that a factor of 3 may be inadequate to account for database deficiencies.

Human Equivalences

Human equivalences are said to reflect adjustments from a less than continuous exposure to continuous exposure, such as might occur in a residence. That assumes continuous exposure although few people are in their homes 24 hours/day. The human equivalence factor is supposed to involve an adjustment from exposures 5 days/week to exposures 7 days/week by multiplying by 5/7 or from 8 hours/day to 24 hours/day when experimental exposures (as in animal studies) are less than continuous. For human occupational exposures, a 10/20 factor is applied to accommodate an increased respiration rate during work; however, when this factor is applied, the adjustment to a 24-hour day is not applied, but the adjustment to a 7-day week is. For oral exposures but apparently not for inhalation exposures, there is an allometric adjustment for body-weight differences. Those considerations are in accord with EPA policy but are far from intuitive and should be summarized in the document where they are applied in the tables. The draft's Figure 5-7 clearly presents that approach in estimating cancer risk, but the figure does not apply to risk posed by inhalation. It is sometimes difficult to see how the "human equivalence" factor is determined for a particular study, and some rationale for its calculation would increase understanding of EPA's approach. For example, the adjustment for the Mattsson et al. study is not described, but it appears to be an adjustment from exposures 6 hours/day 5 days/week to 24 hours/day 7 days/week.

GRAPHICAL PRESENTATION OF REFERENCE VALUES

The draft IRIS assessment provides graphical presentations of noncancer reference values for tetrachloroethylene (Figures 5-1, 5-2, and 5-4). One figure (Figure 5-1) illustrates reference values based on different neurotoxicity studies and two figures (Figures 5-2 and 5-5) compares EPA's selected reference value based on neurotoxicity with other reference values based on other noncancer effects. The committee strongly supports the use of such graphical aids. In general, the approach is intended to make it clear which uncertainty factors were applied, to which studies they were applied, and the effects of particular assumptions. However, the figures in the draft document fail to accomplish that. The shading used in the legend for the figures does not match the shading in the figures, so it is impossible to determine which uncertainty factors were used. By including a small number of studies, the figure on neurotoxicity sample reference values (Figure 5-1) also misses an opportunity to show the degree to which the literature converges on a limited range of sample values. Convergence of estimated values from studies that are methodologically sound, even if they are not listed as key, would support the RfC proposed by EPA.

To synthesize the literature, the committee considered a graphical approach that shows how sample reference values that might be derived from the

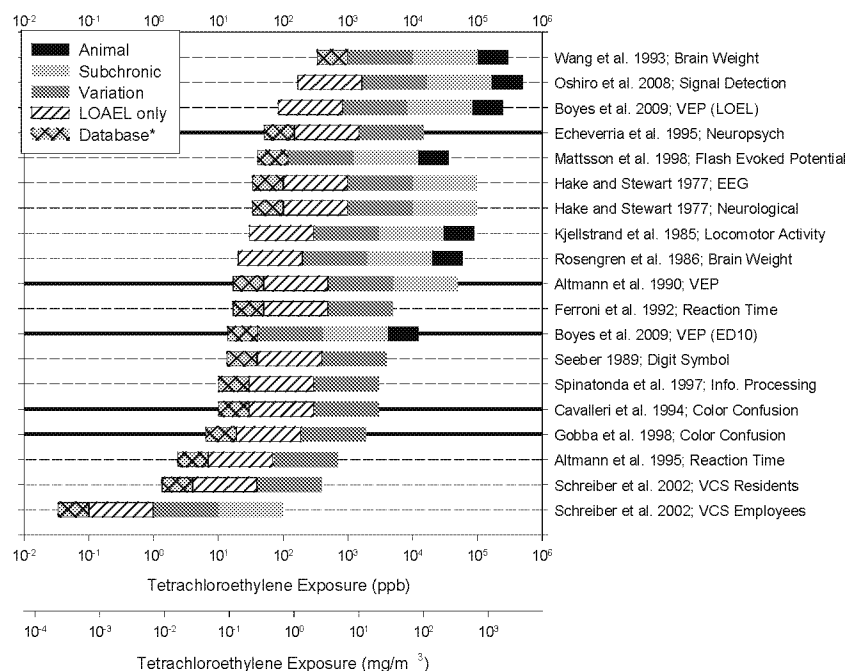


FIGURE 10-1 Distribution of sample reference values. Each horizontal bar represents a single study. Thick, horizontal lines represent studies identified by the committee as most applicable to the development of an RfC. The right end of a bar is at the “point of departure” and is based on concentrations used in the referenced study after conversion to “human equivalencies” or, in the case of animal studies, after adjustment for continuous exposure. Uncertainty factors are illustrated in different shadings: a factor of 3 if it is necessary to extrapolate from animals to humans (black); a factor of 10 if it is necessary to extrapolate from acute or subchronic exposure to chronic exposure (light gray); a factor of 10 for individual variation to account for sensitive individuals (dark gray); a factor of 10 if the study did not contain a NOAEL (diagonal lines) and a factor of 3 for uncertainty in the data base as applied by EPA (light gray, cross-hatched). *A maximum total uncertainty factor of 3,000 was applied for the purpose of this exercise. Where this might be exceeded, the maximum was achieved by omitting the “database” uncertainty so that other uncertainties could be visualized. The committee has recommended that EPA review the uncertainty factors to ensure that they are appropriately explained and used consistently, so some of the individual values used here could be subject to change. In some cases, EPA might judge that the total uncertainty exceeds 3,000 and would, therefore, not use that study to derive a sample reference value. Source: Graphic developed by M. Christopher Newland.

different studies of neurotoxicity compare with one another (see Figure 10-1). That was done by using the studies described in the draft and two studies (Oshiro et al. 2008; Boyes et al. 2009) published since the draft was written. The

approach enables the visualization of the range of concentrations studied, the identification of clusters of studies, and the isolation of especially low or high reference value estimates that might be derived from a particular study. The figure includes seven data points derived from animal studies (identified by a black bar on the right), three from controlled human exposures (identified by a light gray bar and the absence of a black bar), and studies of environmental or occupational exposures. The convergence of sample reference values into clusters would confer confidence on the use of a critical study if other studies led to similar conclusions.

The points of departure for the pre-2004 studies came from Tables 4-4 and 5-2 of the draft document, so they were human adjusted equivalent concentrations or, in the case of animal studies, adjustments for continuous exposures as appropriate. Uncertainty factors were based on how they are typically applied (see pp. 5-11 onward in the draft) even when the committee disagreed with their application. For example, the committee recommends an uncertainty factor different from that applied by EPA for the Schreiber et al. (2002) study. EPA applied an uncertainty factor of 10 to the Schreiber et al. results to extrapolate from “subchronic to chronic exposure,” but the study involved long-term environmental exposure of day-care workers, so the committee believed that this uncertainty factor not necessary. EPA’s factor of 10 was retained in the graphical display, and the RfD calculated for occupational exposure by using this factor appears unusually low.

Studies published since the EPA draft was written are also included in Figure 10-1. One study (Oshiro et al. 2008) identified a LOAEL of 500 ppm (acute). The dependent measure was a signal-detection task. Uncertainty factors for the study would include a factor for extrapolation from animals to humans (3), one for extrapolation from acute to chronic (10), one for sensitive populations (10), one for absence of a NOAEL (10), and the routine one for database uncertainties (3)—for a total uncertainty factor of 9,000. For the purposes of this exercise to show the full database, a maximum total uncertainty factor of 3,000 was applied. The committee has recommended that EPA review the uncertainty factors to ensure that they are appropriately explained and used consistently. In some cases, EPA might judge that the total uncertainty exceeds 3,000 and would, therefore, not use that study to derive a sample reference value. In the graph, the total uncertainty factor was reduced to 3,000 by not showing the uncertainty factor of 3 for database uncertainties. The second study (Boyes et al. 2009) reported a LOAEL of 250 ppm for VEPs evoked by a grid of vertical bars; this is similar to the contrast-sensitivity task. The same factors used in the Oshiro et al. study would be applied here, so a total uncertainty factor of 3,000 (similarly reduced from 9,000) was applied to calculate a sample reference value of 83.3 ppb. The Boyes et al. study also used PBPK modeling to estimate the shape of the relationship between brain concentration and VEP. An ED_{10} , the brain concentration that produced a 10% change in VEP (the last figure in the Boyes et al. paper), was estimated. To estimate a point of departure from the ED_{10} , the exposure concentrations, in parts per billion, that would produce this

brain concentration were estimated by back-calculating from relationships between brain and atmospheric concentrations in the authors' Figures 1 and 2. An ED_{10} of 0.687 mg/mL comes from their Figure 7. From Figure 1, it can be estimated that the brain:blood ratio is 33:12 (at peak), so 0.687 mg/L in brain corresponds to 0.25 mg/L in blood. From Figure 2, it can be estimated that 50 ppm in air corresponds to a peak (and near asymptote) of 1 mg/L in blood. Therefore, the blood tetrachloroethylene concentration of 0.25 mg/L should result from 12.5 ppm in air. The committee recognized that those are rough estimates that assume linearity and that a more precise estimate could be obtained with modeling. The estimate is included here only as an illustration. The 12.5-ppm point of departure yields a sample reference value of 14 ppb after application of uncertainty factors for extrapolation from animals to humans (3), acute exposure (10), variation in sensitivity (10), and database uncertainty (3). It is unclear whether an uncertainty factor should be applied for the absence of a NOAEL in the study.

Some observations can be made from the figure. The majority of sample reference values are centrally clustered, but there is a wide spread to both the lower and higher ends. Although the overall range of the 19 sample values is 0.03-333 ppb (0.0002 - 2.6 mg/m³), it is reduced to about 6 to about 50 ppb (0.04 - 0.34 mg/m³) when restricted to the five strongest studies. EPA's RfC of 2 ppb (0.016 mg/m³) calculated on the basis of the Altmann et al. (1995) study falls below the range and is higher than only the two other human studies, which were conducted by Schreiber et al. The Altmann et al. (1995) and Schreiber et al. (2002) studies are discussed and critiqued elsewhere, where it is noted that the makeup of the critical comparison groups is confounded and that this makes it difficult to attribute differences seen in dependent variables to tetrachloroethylene. The figure enhances transparency by showing how studies converge on a range of reference value estimates and how the study or studies selected as the one(s) to be used for establishing the final RfC compares with other studies.

The three studies that yield sample reference values above 50 ppb are the ones that identified effects at relatively high exposure concentrations because the end points were relatively insensitive or, like in the Oshiro et al. (2008) study, are of very high quality but used high exposure concentrations, so that a low end of the dose-effect curve was not readily identifiable. While the Boyes et al. (2009) study is considered a critical one by the committee, the sample reference value based on the LOAEL from the study (as opposed to the ED_{10}) was considered to have too much uncertainty associated with it to be used as a point of departure. The consistency in the middle ranges among epidemiologic studies and controlled-exposure human studies, as well as in animal studies, provides support for points of departure in these ranges. Despite the use of different exposure regimens and concentrations among animal studies, human chamber studies, and occupational and environmental studies, there is a reasonable coherence in the sample reference values. Finally, to keep the maximum uncertainty factor to 3,000, the "database" factor of 3 was omitted from four estimates for the pur-

poses of the exercise in Figure 10-1. Reinstating this factor would not substantively change the conclusion about the consistency in reference concentrations.

The graphical display in Figure 10-1 shows a distribution of sample reference values based on neurotoxic effects observed in epidemiologic studies, controlled human experiments involving healthy volunteers, and animal experiments involving different species. Exposure ranged from chronic to acute. The studies involved different neurotoxic end points that are differentially sensitive to tetrachloroethylene exposure. Whereas uncertainty factors applied to a point of departure adjust uncertainties specific to their corresponding studies, the collective distribution of reference values provides an overarching measure of uncertainties, weight of evidence, sensitivities, and other sources of variation among different studies.

This approach could also be applied to EPA's other graphical presentations of reference values based on other noncancer end points. Such an approach would allow organ-specific reference values to be put in context with one another. For example, the degree to which sample reference values for an organ system cluster, or fail to do so, might be viewed as evidence of the degree to which different studies tap similar toxic mechanisms, kinetics, end points, or other important characteristics of a chemical.

Cancer Risk Estimates for Tetrachloroethylene

The Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment of tetrachloroethylene provides the agency's assessment of the potential human health effects of exposure to the chemical. For cancer, EPA provides a characterization of the weight of evidence of human carcinogenicity and quantitative estimates of inhalation unit risks and oral slope factors. A unit risk is the upper bound of excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of $1 \mu\text{g}/\text{m}^3$ in air. An oral slope factor is the upper bound, approximating a 95% confidence limit, of the increased cancer risk from lifetime exposure to an agent; it is usually expressed as a proportion (of a population) affected per milligram per kilogram per day. For tetrachloroethylene, EPA proposes a range of inhalation unit risks of 2×10^{-6} to 2×10^{-5} per microgram per cubic meter and a range of oral slope factors of 1×10^{-2} to 1×10^{-1} per milligram per kilogram per day. These ranges reflect the application of three physiologically-based pharmacokinetic (PBPK) models to the same data. This chapter discusses how those cancer risk estimates were determined by EPA.

CANCER CLASSIFICATION

EPA asked for an evaluation of whether conclusions it has drawn in the draft IRIS assessment are consistent with its cancer guidelines (EPA 2005a), specifically with regard to its characterization that tetrachloroethylene is "likely to be a human carcinogen by all routes of exposure." Box 11-1 presents EPA's guidelines for determining such a classification.

The committee considered those guidelines, and guidelines for the other descriptors, and concluded that EPA has documented that its conclusion has been drawn from the results of bioassays that found increased incidences of hepatocellular tumors, hemangiosarcomas, mononuclear-cell leukemia (MCL),

and renal tumors in laboratory animals and to a lesser extent from epidemiologic evidence. EPA's decision to characterize tetrachloroethylene as likely to be a human carcinogen as opposed to "carcinogenic to humans" appropriately reflects that there could be deficiencies or potential inaccuracies in interpretation of the data. Some of those possible deficiencies and inaccuracies are discussed below for each of the data sets.

BOX 11-1 EPA Cancer Guidance for Concluding a Chemical Is Likely to Be Carcinogenic to Humans (EPA 2005)

This descriptor is appropriate when the weight of evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "Carcinogenic to Humans." Adequate evidence consistent with this descriptor covers a broad spectrum. As stated previously, the use of the term "likely" as a weight of evidence descriptor does not correspond to a quantifiable probability. The examples below are meant to represent the broad range of data combinations that are covered by this descriptor; they are illustrative and provide neither a checklist nor a limitation for the data that might support use of this descriptor. Moreover, additional information, e.g., on mode of action, might change the choice of descriptor for the illustrated examples. Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments;
- an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

Mononuclear Cell Leukemia

An increased incidence of MCL in F344 rats has been reported in two bioassays. The biological significance of these increases was debated by the committee because increases were observed only in one strain of rat, which is known to have a high background incidence of MCL. Control data on F344 rats indicate background rates of MCL ranging from 7.9-52.5% in males and 2.1-24.2% in females (Thomas et al. 2007), which make it difficult to interpret the biological significance of increases observed in two studies from different laboratories (NTP 1986; JISA 1993) because of the lack of information on mode of action. Statistical methods, such as survival data analysis, which incorporate data from multiple dose groups simultaneously for dose-response analysis rather than pair-wise comparison should be explored to aid in interpretation. For example, as noted in Chapter 8, Thomas et al. (2007) have made a case that using life-table analysis to examine the MCL data provide an improved approach for interpreting the significance of a dose-response for a possible carcinogenic effect. They judged that there was a positive association between tetrachloroethylene and MCL in the NTP study when such criteria were applied, but recommended a weight-of-evidence evaluation be performed before drawing conclusions. The committee observed that the data showed inconsistency in statistical significance between sexes and uncertainty about the shape of the dose-response curve, especially in the lower range of the NTP study. There is some support from epidemiologic studies which suggest an association between lymphoma and tetrachloroethylene, but the data were relatively weak and inconsistent. A difficulty with interpreting the findings is differences in opinion about the biological concordance between MCL and human lymphohematopoietic cancer. Some members judged that similarities between human natural killer large granular lymphocyte leukemia and rat MCL, as well as mechanistic studies the committee recommended be added to EPA's assessment, are adequate to assume human relevance, whereas others believe more research is needed to establish the relevance. In addition, there was little information on a mode of action for how tetrachloroethylene increases MCL, so it was not possible to distinguish whether exposure to tetrachloroethylene results in initiation of new tumors or enhances the ongoing expansion or promotion of existing tumors.

Hepatic Cancer

Evidence for a statistically significant increase in hepatic tumors was observed in male and female mice after oral or inhalation exposure. Like MCL, the biological significance of these increases was debated by the committee because B6C3F₁ mice have a high background incidence of hepatic cancer (about 20%). However, the findings were reproduced among several studies and conducted in different laboratories and showed a dose-response relationship. There is also fairly substantial information for characterizing potential modes of action for hepatic tumor formation relative to the data available on MCL and renal cancer.

(See Chapters 6 and 7 and the section below on Mode of Action Analysis.) While the committee recommended that EPA revise its presentation of the mode of action evidence for tetrachloroethylene-related hepatic cancer to clarify its position, the majority of the members agreed with EPA that the mode of action is complex and remains to be established. These members also agreed there was insufficient evidence to rule out human relevance. One member objected to these conclusions and the committee's support of using liver cancer to quantify risk. He concluded that in the absence of evidence of other contributing modes of action, the evidence is sufficient to conclude that the mode of action in mice is predominantly through activation of the peroxisome proliferator activated receptor α , a mode of action he considered to be of little relevance to humans. His arguments are presented in a dissenting statement in Appendix B of the report.

Renal Cancer

Tetrachloroethylene caused a low rate of induction of renal tumors in rats. Although the increases were not statistically significant when compared with concurrent controls, EPA has used historical controls to calculate the chances of two of these rare carcinomas to occur by chance to be less than 0.001. Furthermore, a dose-response trend was shown against the low background and the tumors in the treated rats were malignant whereas the tumors in the controls were not. EPA provided a strong evaluation of the potential modes of action evaluation for tetrachloroethylene-induced kidney cancer. The committee agrees with the agency that mode of action of tetrachloroethylene tumorigenesis is not understood, but that a mutagenic mode of action cannot be ruled out. Thus, kidney tumors observed in tetrachloroethylene-treated rats was considered relevant to humans, even though the epidemiologic evidence of an association is weak (see Chapter 7).

SELECTION OF TUMOR TYPE FOR QUANTITATIVE ASSESSMENT

The committee was unable to reach consensus on the selection of the critical cancer end point. The majority of members judged that the uncertainties associated with MCL (particularly the high background incidence, uncertainty about the dose response, and poor understanding of mode of action) were too great to support using the data over that of hepatic or renal cancer for determining quantitative estimates of risk. These members judged that the use of the MCL data could only be justified if it is EPA's policy to choose the most conservative unit risk when considering a range of options, but that such justification should be distinguished as a policy decision and not a scientific one. They believe that a more scientifically defensible approach would be to use the data set with the least uncertainty, rather than the data set that yields the most conservative estimate of risk. In their estimation, the hepatic cancer data would have

the least uncertainty associated with it, followed by kidney cancer and MCL. The comparison of risk estimates presented in the draft IRIS assessment indicates that a unit risk based on hepatic cancer would be approximately eight-fold less than the estimate based on MCL. A unit risk based on kidney cancer would be five-fold less.

Other members judged that the MCL data should be used for cancer risk estimation. Their opinions were based on the observation that reproducible, statistically significant increases in MCL in male and female rats above the background incidence of MCL were found, and that MCL was the cancer end point with the highest magnitude of response. These members believe that use of the most sensitive response to quantify cancer risk decreases the uncertainty associated with potential differences in metabolism and susceptibility to tetrachloroethylene across exposed populations. They concluded that additional statistical analyses of the dose-response data and the addition of supporting mechanistic information identified by the committee would strengthen existing support for MCL in the draft assessment.

MODE-OF-ACTION ANALYSIS

EPA included mode of action (MOA) analyses for cancer in its draft IRIS assessment (Section 4.4.4, pp. 4-16 to 4-35, for the liver and Section 4.5.4, pp. 4-42 to 4-51, for the kidney). EPA's cancer guidelines present a framework for judging whether available data support a hypothesized MOA of an agent. The application of the framework is best demonstrated in EPA's MOA analysis for renal cancer (see Chapter 7). For hepatic cancer, the committee found that the assessment relies too heavily on generic information on peroxisome proliferators and needs to be focused on tetrachloroethylene and its metabolites.

Chapters 6 and 7 provide more specific guidance on how to improve the presentation of the MOA evidence on tetrachloroethylene-induced hepatic and renal cancer. In general, the committee observes that discussion of MOA¹ analy-

¹There was some disagreement among the committee members on what constitutes "modes of action" and "key events." In Section 4.4.4 of the draft IRIS assessment, EPA discusses several "topics" relevant to the MOA for hepatic toxicity, including metabolism, receptor activation, genotoxic effects, and nongenotoxic effects. EPA's presentation treats those topics as separate MOAs, but metabolism is presented as a key event or a component of multiple modes of action. Some committee members judged that that treatment was appropriate as an introduction to a discussion of multiple modes of action and was consistent with EPA guidelines. Other members judged that although early key events may occur in different pathways, they converge to produce one effect; these members hold the view that there is one MOA for an observed effect for which there are a number of specified key events (early key events may be derived from a series of pathways). Despite those differing viewpoints, all members of the committee agreed that more focused analyses of the available evidence are necessary to support hypothesized MOAs.

ses would be improved by including the proposed temporal sequence of hypothesized tetrachloroethylene-associated key events (possibly as a diagram). Transparency would be improved by presenting the details of experimental results in tabular form, including the chemical (tetrachloroethylene or specific metabolite), species, strain, sex, dose, route and duration of exposure, and experimental outcome or end point. That would allow the reader to follow the evaluation of the relative potency of tetrachloroethylene, or its metabolites, in inducing both key events and tumors and to consider species and strain differences with respect to the events and tumor formation. Other data relevant to the evaluation of hypothesized MOAs should be included. The advantage of such a presentation is that it makes explicit the consideration of the timeline of key events in the context of dose, concordance or lack of concordance between early and late events, and the relative contribution of chemical-specific data compared with generic information on categories of chemicals. That should be done for each hypothesized MOA. Even if the data are insufficient to support hypothesized MOAs, the exercise can be used to identify critical data gaps and to inform the direction of future research.

A general difficulty that the committee encountered in reviewing the MOA analyses is the presentation of conclusions without sufficient supporting evidence or reference to prior discussions of the evidence. Much of the experimental evidence was evaluated in other sections of the draft and presumably formed the basis of statements in the MOA considerations. To make the analyses clear, some reiteration of the evidence is needed in discussions of strength, consistency, and specificity of association of the tumor response with key events; dose-response relationships; temporal associations; and biologic plausibility. Coherence of the database is necessary for characterizing the evidence supporting a MOA. The analysis needs to take into account concordance of dose-response relationships between hypothesized key events and end events and to recognize that key events are necessary but might not be sufficient (in their own right) to induce the adverse outcome.

AGE-DEPENDENT ADJUSTMENT FACTOR

Section 6.2.2.1 of the EPA draft (p. 6-24) states that “age adjustment factors for early life exposures as discussed in the *Supplemental Guidance for Assessing Susceptibility for Early-Life Exposure to Carcinogens* (U.S. EPA 2005b) are not recommended because little evidence exists to indicate that tetrachloroethylene or its oxidative metabolites directly damage DNA, information about genotoxicity of glutathione (GSH) metabolites in cell assays other than *Salmonella* or in in vitro experiments are lacking, and the MOA for tetrachloroethylene has not been established.” In addition, the assessment reasons that “although a mutagenic MOA would indicate increased early-life susceptibility, there are no data exploring whether there is differential sensitivity to tetrachloroethylene carcinogenicity across life stages.” The committee’s recommendations for amending sections on

genotoxicity and MOA considerations would also strengthen the arguments made by EPA with regard to the need for age-adjustment and low-dose extrapolations. The committee concluded that several metabolites of tetrachloroethylene are clearly genotoxic: *S*-(1,2,2-trichlorovinyl) glutathione (TCVG), *S*-(1,2,2-trichlorovinyl)-L-cysteine, *N*-acetyl-*S*-(1,2,2-trichlorovinyl)-L-cysteine (N-Ac-TCVC), tetrachloroethylene oxide, dichloro-acetic acid (DCA), and chloral hydrate (if it is formed). However, it is questionable whether those metabolites play an important role in the MOA of tetrachloroethylene carcinogenesis in view of their presence in tetrachloroethylene-exposed animals at low or undetectable concentrations and in the absence of convincing evidence of mutagenic and tumor-initiating activity of tetrachloroethylene in vivo. In addition, the committee supports EPA's conclusion that the MOA of tetrachloroethylene is unclear and probably complex. Thus, although the committee agrees that age-dependent adjustment factors for cancer risk should not be applied, given uncertainties with regard to the overall MOA and the biologic relevance of the data on genotoxicity of metabolites of tetrachloroethylene, the rationale for this conclusion should be revisited.

LOW-DOSE EXTRAPOLATION

For cancer risk assessment, EPA relied on the default option of low-dose linear extrapolation to estimate inhalation unit risks and oral slope factors for tetrachloroethylene. EPA describes low-dose linear extrapolation in detail (in Section 5.4.4 of the draft). It entails three steps. First, a dose-response model, typically a mathematical function in the absence of MOA information, that appropriately fits observed data within the experimental data range must be identified. Second, a point of departure (POD) (a benchmark dose or benchmark concentration) along the fitted dose-response model is determined; it corresponds to an exposure that typically induces about 5-10% extra risk above the control's response rate. Then the associated extra cancer risk is divided by the POD to yield a unit risk or a slope factor.

In the draft IRIS assessment, EPA illustrates low-dose extrapolation with six datasets, hepatocellular adenoma or carcinoma in male and female mice (JISA 1993), hemangiosarcoma in male mice (JISA 1993), MCL in male and female rats (JISA 1993), and renal tumors in male rats (NTP 1986). EPA considered multistage models as well as multistage Weibull models for dose-response modeling in conjunction with the dose metric of total metabolism and administered concentration but presented results only of multistage models. It justified the use of the multistage model (p. 5-50) on the basis that MOA information is lacking and that the model has "some parallelism to the multistage carcinogenic process and it fits a broad array of dose-response patterns. Occasionally the multistage model does not fit the available data, in which case an alternate model should be considered." In the case of hepatocellular adenoma and carcinoma in male mice, hemangiosarcoma in male mice, and MCL in female rats, the multistage model does not fit the data at lower doses, as acknowl-

edged by EPA (Figures 5-8a, 5-10a, and 5-12a). EPA did not explain the possible underlying reasons for low-dose nonlinearity and potential adjustment. EPA considered those poor-fit models acceptable solely on the grounds that statistical tests for goodness of fit were not significant ($p > 0.10$). The committee notes that the lack of significance of goodness-of-fit tests can be the result of a small number of animals in each dose group. For example, by doubling the number of animals per dose group while keeping the incidences of tumor the same as in the original dataset of hepatocellular adenoma and carcinoma in male mice (JISA 1993), we can fit the same model (Table 5-11) to the “larger” experiment. The goodness-of-fit test would reach a p value of 0.04, which suggests a poor fit. Alternatively, if we were to fit a multistage model with a (polynomial) degree of 2 to the original data, the goodness-of-fit test would have a p value of 0.25, which would suggest a better fit than the model chosen by EPA (Table 5-11). Thus, using the goodness-of-fit test to justify a selection of a dose-response model can be misleading. Furthermore, contrary to the statement that “dose-response modeling of the candidate data sets presented no particular difficulties” (EPA 2008, p. 5-69), the benchmark dose software automatically fixed some parameters to zero to obtain convergence in model fitting. For example, in the case of hepatocellular adenoma and carcinoma in male mice, the second order coefficient ($q_2=0$) is fixed but the third order coefficient (term q_3) is not. The criteria under which EPA selected parameters and fixed them was unclear. Also, the parameter q_0 reported in Tables 5-10 and 5-11 should be reported as $1-\exp(-q_0)$ to be consistent with the specification of multistage model in section 5.4.4.1. The committee also notes that the polynomial order used in the multistage dose-response models is limited by the number of dose groups in each experiment; only lower-order multistage models can be fitted, and they are forced to be nearly linear in the low dose range. Therefore, the similarity between the slope of the models and the unit risk taken from the models reflects more on the nearly linear model imposed on the data than the true shape of the dose-response curve. The questionable fitting of a multistage model to some candidate datasets and the insufficient consideration of alternative models in these situations appear to be inconsistent with EPA’s cancer-risk guidelines and can contribute to underestimation of the overall uncertainties.

Once a dose-response model was chosen, EPA carried out the estimation of benchmark concentration with its lower confidence limit (BMCL) at a 10% extra risk (5% in one case). The BMCL is used as a POD for unit risk or slope factor. EPA’s choice, estimation, and presentation of PODs are adequate and clear.

EPA adopted linear low-dose extrapolation, the default option, with several justifications. First, MOA information is insufficient, and support for dynamic models unavailable. Therefore, nonlinear mechanistic models are unavailable for dose-response modeling. Second, because mathematical models are subject to uncertainties for low-dose extrapolation beyond the experimental dose range, linear extrapolation is more conservative than all sublinear (curvilinear) dose-response models. When individual thresholds in the human population are

plausible, wide variation in threshold values implies a curvilinear shape of the dose-response relationship on the average. Thus, linear extrapolation protects susceptible subpopulations (NRC 2009). Third, a few of the candidate datasets, especially EPA's preferred male-rat MCL data, exhibit a linear pattern of dose-response relationships. Whereas those arguments are consistent with EPA's *Guidelines for Carcinogen Risk Assessment*, there is evidence in the candidate datasets that the underlying dose-response relationship can be even supralinear (for example, in female-rat MCL). When that is the case, low-dose linear extrapolation is not conservative. The full range of variation and uncertainty in relation to model choice is not presented, in part because EPA did not consider the possibility of other forms of nonlinear dose-response models, including supralinear, for all candidate datasets.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS, DOSE METRICS, AND INTERSPECIES SCALING

The draft IRIS assessment appears to do a thorough job of reviewing the pertinent scientific literature on the toxicokinetics of tetrachloroethylene. EPA considered several independent efforts to develop physiologically based pharmacokinetic (PBPK) models for tetrachloroethylene and used them to estimate human equivalent doses in terms of environmental exposure and to perform route-to-route extrapolation. In the sections below, the committee reviews EPA's decisions about what PBPK models to use, its choice of dose metrics, and approaches to species extrapolation.

The committee reviewed the original papers describing the selected PBPK models and supporting studies, which in some cases provided the experimental data used to validate model predictions. Evaluation of dose metrics was based on two primary criteria: the ability of the PBPK models to provide discrete estimates of a metric (such as peak blood levels or AUC of the parent chemical or metabolite in blood or target tissue) and the relevance of the parent compound or metabolites to the toxic end point. For cancer, the available evidence suggested that various tetrachloroethylene metabolites were involved or responsible, depending on the end point.

Physiologically Based Pharmacokinetic Modeling Approaches

There have been an unusually large number of independent efforts to develop PBPK models for tetrachloroethylene. EPA is to be commended for its willingness to use the PBPK modeling approach and to explore or test the various published PBPK models for tetrachloroethylene in its risk assessment. EPA used three PBPK models (Rao and Brown 1993; Reitz et al. 1996; Bois et al. 1996). However, there is a notable lack of critical evaluation of the models. Because the most important differences between the models is in prediction of tetrachloroethylene metabolism, there should be more discussion of the pros and

cons of using a population-modeling approach as in the Bois et al. (1996) study vs the other models, which rely more directly on animal in vitro and in vivo data. In particular, there seems to be a divergence between the two approaches particularly in estimating the fraction metabolized after smaller tetrachloroethylene exposures. For example, the recent paper by Chiu and Bois (2006) suggests that much higher fractions (23% of the dose) of tetrachloroethylene are metabolized in humans after low exposure (less than 1 ppm).

Reading the descriptions of previous PBPK modeling efforts makes it clear that it would have been preferable for EPA to pursue development of a “harmonized” PBPK model (as was done for trichloroethylene), which synthesized important aspects of the various models (the use of multiple exposure routes and inclusion of all relevant tissue compartments) into a single model. In connection with this recommendation, it is important to recognize that most PBPK models of tetrachloroethylene (and trichloroethylene) are highly derivative of the PBPK model for methylene chloride published by Andersen et al. (1987). The differences between the tetrachloroethylene models are associated with inclusion or exclusion of routes of exposure and the use of experimental data to select parameters for models and validate model predictions. The approach pursued by EPA, using three PBPK models, is a reasonable alternative for the tetrachloroethylene risk assessment for which the goal is to estimate tetrachloroethylene dosimetry related to inhalation exposure. The population pharmacokinetic modeling approach used in the Bois et al. model empirically estimates metabolism parameter values to provide an adequate fit of observed tetrachloroethylene exposure data. Initial estimates (prior distributions) in the Bois et al. model were obtained from the literature by using many sources, and the final estimates (posterior distributions) were obtained by using a Markov-Chain-Monte Carlo approach.

It would have been preferable to use a single PBPK model. All three of the selected models are adequate for characterizing parent-compound (tetrachloroethylene) dosimetry, but they are not equivalent in characterizing tetrachloroethylene metabolism. There is inadequate justification for the selection of dose metrics for tetrachloroethylene metabolism, particularly in the use of total metabolites as the overall dose metric for cancer. The risk assessment would be improved if more effort were devoted to estimating the fraction of an absorbed tetrachloroethylene dose that enters the GSH pathway and the fraction entering the cytochrome P-450 pathway, which leads to the formation of trichloroacetic acid (TCA). That would permit development of more discrete, rational, and defensible dose metrics (for example, total GSH metabolites vs P-450 metabolites) for cancer end points.

The committee recommends that EPA pursue development of a single “harmonized” PBPK model that includes all routes of exposure (inhalation, oral, and dermal) and all relevant tissue compartments. With regard to metabolic dose metrics, the initial goal should be to predict the fraction of an absorbed tetrachloroethylene dose that enters the GSH pathway (initially forming TCVG) and the fraction that enters the P-450 pathway (eventually leading to TCA forma-

tion). That would permit development of more discrete dose metrics (such as total GSH metabolites vs P-450 metabolites) and should lead to a more rational and defensible selection of dose metrics for the various cancer end points.

Given the incomplete data available for characterizing the GSH pathway, several approaches may need to be adopted that rely on rodent in vitro data, human in vitro data where available, and allometric scaling as needed. For some key reactions, a parallel approach with trichloroethylene metabolism might be considered; in this respect, the approach and recommendations described by Lash and Parker (2001) should be considered and tested with appropriate model simulations. If modeling the GSH pathway is determined to be infeasible, total metabolism can be used as a reasonably conservative dose metric.

The PBPK model could then be built and tested around a combination of blood tetrachloroethylene and TCA concentrations, in vitro metabolism data, and urinary-excretion data for various metabolites (such as TCA, *N*-Ac-TCVC). With a single harmonized PBPK model, the population modeling approach could be used more effectively to estimate a range of V_{\max} and K_m values and compare these posterior distributions with a more robust dataset of blood, in vitro, and urinary-excretion data.

Dose-Metric Selection

The rationale for selection of most dose metrics is clearly explained in the draft IRIS assessment. However, the committee is concerned about the selection of the dose metrics chosen for tetrachloroethylene metabolism. As thoroughly reviewed in the draft, tetrachloroethylene metabolism can be separated into cytochrome P-450-derived oxidative metabolites produced primarily by the liver (the P-450 pathway) and metabolites derived from the initial formation of a GSH conjugate (the GSH pathway) and later reactions in several tissues, including the kidney. The P-450 pathway produces several metabolites, including the biologically persistent metabolite TCA. The P-450 pathway is more closely linked to hepatic cancer in rodent models whereas the GSH pathway appears to be associated more with renal tumors and perhaps leukemia. EPA has chosen not to estimate the flux of metabolism through the GSH pathway and summarizes the rationale for that decision as follows (p. 5-48): "However, the measurements of glutathione-dependent metabolism are from in vitro studies or they are measures of urinary excretion products and are, therefore, not representative of the toxic species in vivo." Instead, the dose metric of total metabolism is used for all cancer end points in which tetrachloroethylene metabolites are implicated. That approach has created several potential problems that are not adequately addressed in the draft. The rationale for excluding the GSH pathway is inconsistent with the use of the three PBPK models, which also use in vitro data (the Reitz model) or urinary-excretion data (the Rao and Brown model) to estimate total metabolism. A fair question to ask is why the use of in vitro data and measures of urinary excretion products was acceptable for the P-450 pathway

but not the GSH pathway. The use of total metabolism as a dose metric reflects primarily the P-450 pathway because of large differences between the pathways in the flux of metabolism. The approach used by the different PBPK models to estimate metabolism and specifically estimation of the key metabolic parameters V_{\max} and K_m varies substantially. Estimation of total metabolite formation in humans with the Reitz model relies primarily on *in vitro* hepatic metabolism data (microsomal metabolism, hence only the P-450 pathway) whereas the Rao and Brown model is validated by urinary excretion of nontetrachloroethylene radioactivity and TCA (also reflective primarily of the P-450 pathway). Although there is less experimental information on the GSH pathway, there are *in vitro* data from two studies that characterize the formation of TCVG in rodents (Dekant et al. 1998; Lash et al. 1998). The Dekant et al. (1998) study also attempted to measure TCVG in human tissues but was unable to detect it. However, their analytic methods appear to be rigorous and to allow estimation of the highest formation rate that could have occurred (still producing undetectable concentrations of TCVG), which would be helpful for risk assessment. A summary of the rates of TCVG formation in the liver in those studies is presented in Table 11-1. These values could be used to estimate the *in vivo* formation clearance of TCVG in the liver and kidney (data available but not included in Table 11-1) with an approach outlined by Houston and Carlile (1997). It would have been valuable if an attempt had been made to estimate the flux of tetrachloroethylene metabolism through TCVG in rodents and compare it with that in humans by using the results of Dekant et al. (1998) as an upper limit of formation rate. The modeling exercise could be strengthened by integrating the human urinary-excretion data reported by Volkel et al. (1998), for example, on detection of *N*-Ac-TCVC but not DCA in tetrachloroethylene-exposed volunteers.

With respect to hepatic cancer, it is debatable whether it is preferable to use a trichloroacetic acid dose metric as opposed to total metabolites. The recent paper by Sweeney et al. (2009) makes a strong argument for the former. However, given the potential role of other P-450 pathway-derived tetrachloroethylene metabolites (discussed in Chapter 6) in hepatic cancer, the use of total metabolites as the dose metric appears justified. In addition, experimental evidence suggests that the toxicity of a directly administered metabolite does not reflect that of the “formed” metabolite (TCA in the case of tetrachloroethylene) even when blood concentrations are comparable (Pang 2009).

The use of total metabolites as a dose metric for renal cancer is not well supported. According to the available data (see Chapter 7), tetrachloroethylene metabolites derived from the GSH pathway are most likely to be the causative agents. Thus, a dose metric that more accurately reflects the flux of metabolism through the GSH pathway would be preferred. For reasons discussed previously, total metabolites constitute essentially a dose metric for the P-450 pathway. The committee encourages EPA to put forth a more thorough effort to develop a TCVG-based dose metric for rodents and possibly humans by using the available data summarized in Table 11-1.

TABLE 11-1 Summary of Data on Hepatic Metabolism of Tetrachloroethylene and Urinary Excretion of Glutathione-Pathway Metabolites

Metabolite Formation	Species			Reference or Initial Substrate Concentrations	
	Rat (nm/mg per hour)	Mouse (nm/mg per hour)	Human (nm/mg per hour)	Lash et al. (1998)	
♂-TCVG (hepatic cytosol)	10.6 ± 2.2	20.7 ± 4.7	nm	2 mM tetrachloroethylene	
♂-TCVG (hepatic microsomes)	8.4 ± 1.1	24.5 ± 3.4	nm	5 mM GSH	
♀-TCVG (hepatic cytosol)	6.2 ± 0.8	16.3 ± 1.3	nm	Dekant et al. 1998	
♀-TCVG (hepatic microsomes)	3.7 ± 0.9	15.6 ± 1.2	nm	3 mM tetrachloroethylene	
♂-TCVG (hepatic cytosol)	5.1 ± 0.7	1.7 ± 0.4	<0.06	5 mM GSH	
♂-TCVG (hepatic microsomes)	nd	nd	<0.06		
♀-TCVG (hepatic cytosol)	1.2 ± 0.5	1.6 ± 0.4	<0.06		
♀-TCVG (hepatic microsomes)	nd	nd	<0.06		
Cumulative urinary excretion	nm/kg		nm/kg		
N'-Ac-TCVC (both sexes)	3.5 – 415		0.65 – 3.01	Volkel et al. 1998	
TCA	1.9 – 66.7		70 – 290	Tetrachloroethylene in vivo	
DCA	0.25 – 5.5		nd	10–40 ppm	

Note: TCA data included for comparison.

Abbreviations: DCA = dichloroacetic acid; GSH = glutathione; N'-Ac-TCVC = N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine; nd = not detected; nm = not measured; TCA = trichloroacetic acid; TCVG = S-1,2,2-trichlorovinyl glutathione.

Interspecies Scaling

The approach used for interspecies scaling is presented in a reasonably clear manner. Figure 5-7 of the draft and the discussion on pp. 5-53 to 5-55 are particularly helpful. The committee's main concern in this regard is how errors in estimating the metabolized fraction affect the extrapolation process.

Extrapolation from Route to Route

EPA has chosen to use the venous-blood area under the curve (AUC) as the route-to-route dose metric for extrapolating an inhalation exposure to a corresponding oral dose. The rationale for this approach is sound and adequately explained in the draft document. However, its implementation raises serious methodologic concerns based on inappropriate use of the selected PBPK models and uncertainties in the fraction of an oral dose of tetrachloroethylene that is metabolized. The three PBPK models used by EPA were specifically formulated and validated against inhalation exposures. There was no attempt to validate model predictions against blood tetrachloroethylene concentrations after oral dosing. To use the PBPK models, EPA has empirically assumed a value of the rate of oral absorption of tetrachloroethylene, which is entered as a constant. That approach is inferior to direct estimation as used in other published PBPK models, such those by Gearhart et al. (1993) and Dallas et al. (1995) (the latter only for rats and dogs). These PBPK models would have been better choices to begin the extrapolation exercise. Better still, a harmonized PBPK modeling approach (recommended earlier in this chapter) would have provided the greatest confidence in the route-to-route extrapolation.

Aside from the use of an appropriate PBPK model (for example, one specifically formulated and validated against oral-dosing data), uncertainty is associated with the dose dependence of tetrachloroethylene metabolism. EPA has assumed that a person will have nine drinking-water events during a day at roughly 2-hour intervals (excluding nighttime). The calculated oral equivalent dose of tetrachloroethylene is 1.1 mg/kg per day or 0.122 mg/kg per dose (that is, the discrete tetrachloroethylene dose received in each drinking-water episode). That oral dose is an order of magnitude lower than those previously used in toxicokinetic studies of tetrachloroethylene. The data from past studies clearly suggest that the fraction of a tetrachloroethylene oral dose that is metabolized is progressively reduced as the dose increases (Pegg et al. 1979; Frantz and Watanabe 1983; Schumann et al. 1980; Dallas et al. 1995). The issue of uncertainty in fractional tetrachloroethylene metabolism and dose was also raised by Reitz et al. (1996), whose PBPK model was used by EPA for route-to-route extrapolation of total metabolites. That raises the serious concern that a much greater fraction of the 0.122-mg/kg dose of tetrachloroethylene is being metabolized than was predicted by the PBPK models used in the risk assessment. The impact of the probable error is that the estimates of the venous-blood AUC of tetra-

chloroethylene shown in Figure 5-3 of the draft are probably overpredicted (that is, a higher oral dose is needed) and the estimates of total metabolites are underpredicted and may affect cancer assessments.

UNCERTAINTY

Cancer risk assessment results in an overarching summary of cancer risk by using a unit risk or a slope factor. In the process of deriving the unit risk or slope factor, uncertainty at every step is propagated into the final estimate. Because of the quantitative nature of the final risk estimates, it is critical to understand the effects of uncertainties on risk estimates both qualitatively and quantitatively. EPA has clearly identified key sources of variation and uncertainty in the process of risk assessment, including human population variation (susceptibility in exposure, metabolism, and response to exposure), low-dose extrapolation (including choice of dose-response models), choice of dose metric, extrapolation from animals to humans (cross-species scaling), and the use of PBPK models for route-to-route extrapolation. EPA's investigation of the effects of uncertainties on risk estimates is qualitative except in dealing with such issues as the choice of dose-response models, the use of PBPK models, and, to a small degree, variation between studies. The following is an appraisal of EPA's uncertainty analysis.

EPA's presentation of the uncertainty analysis is generally transparent and includes sufficient detail. The tabular presentation of choices of study, end points, the approach (models) to extrapolation, and their effects on risk estimates is especially informative and easy to follow. For example, Table 6-3 of the draft summarizes key characteristics of the candidate rodent experiments and associated tumor types. Whereas that form of presentation is helpful, the committee does not agree with all characterizations presented in the table (see earlier discussion about the different cancer end points).

Similarly, Table 6-5 highlights EPA's choices and their effects on the determination of the upper bound of the risk estimate at many critical steps of the risk-estimation process. It also lists EPA's decision and the corresponding justification. Such presentation is effective and should be fully used. In some instances, however, the justification of EPA's choice is debatable. As discussed in Chapter 6, for example, the committee believes that the hepatic-tumor data on male and female mice should also be given strong weight for consideration on the basis of dose-response data. In the case of the choice of dose-response model from among the multistage, Weibull, log-probit, and log-logistic models, one justification for using a multistage model was the relatively small variation in unit risk among the four models (a factor of 1.4). However, that narrow variation was shown only in the male-rat MCL data. The MCL data exhibit a nearly linear dose-response relationship and hence attenuate the difference among the four models. If EPA would consider other bioassay or tumor sites (such as hepatic tumors in female mice or MCL in female rats) that show a somewhat more

nonlinear shape of the dose-response relationship, the variation in unit risk calculated by the models would be much greater. Even in the case of MCL in male rats, the risk obtained by linear extrapolation to 1.5×10^{-5} mEq/kg per day varied by up to several orders of magnitude among the same four models (Table 5B-2). Therefore, choosing a multistage model on the basis that risks with other models at a POD are similar is difficult to justify.

More detail would have been helpful in a few of EPA's analyses of uncertainties. For example, EPA's assessment of uncertainties under different model forms (multistage, Weibull, log-probit, and log-logistic) used bootstrap simulations. The results show variation in extra risk spanning orders of magnitude at the low dose of 1.5×10^{-5} mEq/kg per day (bootstrap mean, 9.172×10^{-7} to about 1.078×10^{-3} in Table 5B-2) among the models despite their comparable goodness of fit to the dataset on MCL in male rats. Details about the bootstrap methods and scheme would facilitate appropriate understanding of the bootstrap distributions. For example, what was the number of bootstrap replications? What bootstrap method was used to simulate the distribution of extra risk? The committee views EPA's consideration of uncertainty due to different forms of the dose-response relationship highly valuable, and it encourages EPA to extend such quantitative evaluation to all candidate datasets so that a fuller array of uncertainties can be assessed.

The committee notes that EPA discusses uncertainties in detail. However, the discussion typically focuses on individual sources without an in-depth illustration of the propagation of the uncertainties and their cumulative effect on the final risk estimate. That limitation is in part the result of qualitative treatment of uncertainties in many instances, notably concerning MOA, the choice of bioassay, and human variations. New methods that allow probabilistic quantification of the overarching uncertainty and of the variation in the final risk estimate are emerging (see Chapter 12). The capability to quantify the full range of overarching uncertainties associated with risk estimates facilitates separation of the science of risk assessment from risk-management decision-making. The committee encourages EPA to consider recommendations in *Science and Decisions* (NRC 2009) regarding uncertainty and variability.

Moving Beyond the Current State of Practice

The committee found that the Environmental Protection Agency (EPA) draft Integrated Risk Information System (IRIS) assessment could be improved in several ways. Such changes are not necessary for completing the current assessment but should be considered when tetrachloroethylene is re-evaluated. They include improvements in the presentation and organization of information, addition of transparency in documenting procedures used for identifying and selecting studies, and the use of evolving approaches to uncertainty analysis. Guidance in many of these areas is provided in a recent National Research Council (NRC 2009) report *Science and Decisions*, which discusses advancing risk assessment practices.

ORGANIZATION AND APPROACH

There is a vast amount of literature on tetrachloroethylene, and drafting of the IRIS assessment was hampered by the need to manage such a large volume. EPA should consider ways of reorganizing the document to streamline the presentation of data and analyses. The current organization requires that some information be duplicated in various places. Part of the document also appears to be targeted to controversies in interpretation of some aspects of the data. In several instances, the committee found that EPA had spent more time in debunking others' positions than in bolstering its own arguments.

Although the draft provides a comprehensive review of the available data, it is not clear whether studies were evaluated case by case or a consistent set of criteria were applied. To ensure consistent and transparent analysis of the data, criteria for identifying, analyzing, and selecting studies should be established in advance to guide the assessment in focusing on the most relevant studies. Study design and methods are the most important factors in study selection. Other fac-

tors, such as exposure considerations and outcomes, will also play a role in selection.

Consideration of the quality of an assessment is predicated on not only its content but the process by which it was prepared. There should be a preassessment discussion of problem formulation and issue identification that indicates the extent of reliance on previous reviews, the focus of the future effort, and the specific issues on which the assessment is likely to be focused. (Guidance on the design of a risk assessment in its formative stages is provided by the NRC [2009].) That would serve as a basis for soliciting external multidisciplinary input at an early stage in such critical matters as mode of action and evaluation of information on specific end points (including both toxicologic and epidemiologic data). It would include a priori delineation and weighting of criteria for evidence of hazard and options analysis for dose-response assessment and associated uncertainties. Attention to specifying evaluation criteria and the options considered is expected to contribute considerably to transparency in the separation of science judgment from science-policy choices.

To increase transparency, accountability, and defensibility and to improve the content and process of assessments, the committee offers the following recommendations regarding future assessments of tetrachloroethylene:

- The nature of, timeframe for, and extent of consideration of relevant data should be clearly framed and stated (for example, standard searching of identified electronic sources with criteria specified, cutoff date past which no additional data were considered, and identification of current studies by reviewers).
- Exclusion criteria for particular studies should be clearly identified and explained (for example, unpublished or published after a particular date). In particular, there should be description of steps taken to ensure that studies identified after the original search were selected without bias from the totality of the available data.
- The methods used for qualitative characterization of uncertainties should be clearly identified, explained, and documented. Qualitative assessment of uncertainty involves (WHO 2008) evaluation of the level of uncertainty of each specified source according to a scoring method, identification and description of the major sources of uncertainty, appraisal of the knowledge base associated with each major source of uncertainty, identification of controversial sources of uncertainty, evaluation of the subjectivity of choices of controversial sources of information, and iteration until the output reflects the current state of knowledge.
- The specific nature of the process of preparing and reviewing the assessment—including identification of authors and reviewers, timeline and nature of peer input, consultation, and peer review—should be set forth.

UNCERTAINTY ASSESSMENT

Scientific Needs

Beginning as early as the 1980s (NRC 1983), expert scientific advisory groups have been recommending that risk analyses include a clear discussion of the uncertainties in risk estimation. The National Research Council (NRC 1994; 2009) stated the need to describe uncertainty and to capture variability in risk estimates. The Presidential/Congressional Commission on Risk Assessment and Risk Management (PCCRARM 1997) recommended against a requirement or need for a “bright-line,” or single-number, level of risk. Regulatory science often requires selection of a limit for a contaminant, but the limit always contains uncertainty as to how protective it is. Explicit quantification of uncertainty enables decisions regarding degree of protection to be made in the policy arena rather than buried among assumptions of a technical analysis. Risk characterization became EPA policy in 1995, and the principles of transparency, clarity, consistency, and reasonableness are explicated in the 2000 *Risk Characterization Handbook* (EPA 2000). Criteria for transparency, clarity, consistency, and reasonableness require analysts to describe and explain the uncertainties, variability, and known data gaps in a risk analysis and imply that decision-makers should explain how they affect resulting decision-making processes (EPA 1992, 1995, 2000).

On numerous occasions, the National Research Council has explicitly called for the use of probabilistic risk assessment (NRC 2006b, 2007). In 1983, it formalized the risk-assessment paradigm that includes dose-response analysis as a key component (NRC 1983). In 1989, it recommended that EPA consider the distribution of exposure and sensitivity of response in the population (NRC 1989). In 1991, it stated that when assessing human exposure to air pollutants, EPA should present model results with estimated uncertainties. In 1993, it recommended that EPA thoroughly discuss uncertainty and variability in the context of ecologic risk assessment (NRC 1993). In 1994, in a major review of risk-assessment methodology, it stated that “uncertainty analysis is the only way to combat the ‘false sense of certainty,’ which is *caused* by a refusal to acknowledge and (attempt to) quantify the uncertainty in risk predictions” (NRC 1994). And in 2002, it suggested that EPA’s estimation of health benefits was not wholly credible, because the agency failed to deal formally with uncertainties in its analyses (NRC 2002).

EPA’s Science Advisory Board (SAB) has made recommendations similar to those of the National Research Council. It urged EPA to characterize variability and uncertainty more fully and more systematically and to replace single-point uncertainty factors with a set of distributions by using probabilistic methods (EPASAB 2007). EPA has developed numerous internal handbooks on conducting quantitative analysis of uncertainties in various contexts (e.g., EPA

1995, 1997, 1998, 2000, 2001). In 2009, it provided a detailed overview of the current use of probabilistic risk analysis in the agency (including 16 detailed case-study examples), an enumeration of the relevance of probabilistic risk analysis to decision-making, common challenges faced by decision-makers, an overview of probabilistic risk-analysis methodology, and recommendations on how probabilistic risk analysis can support regulatory decision-making. EPA's National Exposure Research Laboratory has recently explored methodologic issues in dealing with uncertainty quantitatively when air-quality, exposure, and dose models are coupled (Ozkaynak et al. 2008).

There are numerous texts on analysis of uncertainty (e.g., Morgan and Henrion 1990; Cullen and Frey 1999; Vose 2008). The World Health Organization (WHO) has recently released guidance on qualitative and quantitative methods of uncertainty analysis in the context of exposure assessment (WHO 2008). Its guidelines have been used by EPA to support uncertainty assessments related to exposure to and health effects of criteria pollutants under the National Ambient Air Quality Standards. Hence, the framework is a general one. In particular, WHO proposed guiding principles that are adapted as follows:

- Uncertainty analysis should be an integral part of the assessment.
- The objective and level of detail of the uncertainty analysis should be based on a tiered approach and be consistent with the overall scope and purpose of the assessment.
- Sources of uncertainty and variability should be systematically identified.
- The presence or absence of moderate to strong dependence of one input on another should be discussed and appropriately accounted for.
- Data, expert judgment, or both should be used to inform the specification of uncertainties in scenarios, models, and inputs.
- Sensitivity analysis should be an integral component of the assessment.
- Uncertainty analyses should be fully and systematically documented in a transparent manner, including quantitative aspects pertaining to data, methods, inputs, models, and outputs; sensitivity analysis; qualitative aspects; and interpretation of results.
- The results of the assessment, including uncertainty, should be subject to an evaluation process that may include peer review, model comparison, quality assurance, or comparison with relevant data or independent observations.
- Where appropriate for an assessment objective, assessments should be iteratively refined to incorporate new data and methods to reduce uncertainty and to improve the characterization of variability.
- Communication of assessment uncertainties to stakeholders should reflect the needs of different audiences in a transparent and understandable manner.

Decision-Making Context for Use of Uncertainty Assessment

EPA decision-makers face scientifically complex problems that entail uncertainty. A risk assessment includes exposure assessment, dose-response assessment, and risk characterization. Methods for quantifying uncertainty in exposure assessment are well accepted and widely applied (e.g., Cullen and Frey 1999). Risk can be characterized for a population (for example, the expected number of excess cancers) or an individual (for example, the incremental lifetime risk of excess cancer). The need for characterization of uncertainty in risk characterization is supported by numerous National Research Council studies (for example, NRC 1994). The decision context of risk assessment includes setting priorities for the activities of the assessment and development of data for the assessment to characterize and, where possible, reduce uncertainty and managing risk. Decision-makers often want to know who is at risk, the magnitude of risk, and tradeoffs between risk-management alternatives. Examples of specific questions that decision-makers may ask include the following (Bloom et al. 1993; Krupnick et al. 2006):

- How representative is the estimate (for example, what is the variability around an estimate)?
- What are the major gaps in knowledge, and what major assumptions are used in the assessment? How reasonable are the assumptions?
- Is it likely that additional data collection and research would lead to a different decision? How long would it take to collect the information, how much would it cost, and would the resulting decision be substantially different?

Moving Beyond the Current State of Practice

EPA's assessment of tetrachloroethylene follows a traditional approach for developing "cancer slope factors" and "hazard indexes" that take into account uncertainties qualitatively and through uncertainty factors. Although EPA claims to have introduced a new method for uncertainty analysis in the context of the dose-response assessment of tetrachloroethylene, in fact the only differences between the draft IRIS assessment for tetrachloroethylene and those of other chemicals are the consideration of multiple end points and the limited use of bootstrap simulation for only a portion of uncertainties. The various alternative dose-response estimates developed represent inter-end-point variability, not uncertainty.

The well-accepted default-based approach to developing dose-response relationship estimates leads to point estimates, not distributional ranges. The choice of point estimates is based on default assumptions regarding uncertainty factors and default inference methods for fitting and interpretation of dose-response functions. Therefore, such estimates do not depict uncertainty quantita-

tively in conjunction with the final result, and they are based on assumptions that may mix policy judgments about degree of protection and scientific goals of developing a best estimate. Thus, the state of practice does not fully meet the spirit of principles, guidelines, and recommendations that have accrued over the years from such science advisory bodies as the EPA's SAB, WHO, and most recently the National Research Council (NRC 2009). Today, the approach that EPA has taken is considered to be the best practice but not a state-of-the-art practice. For example, although uncertainty factors are used to account for such issues as extrapolation from subchronic to chronic exposure, interspecies extrapolation, and adjustments from lowest-observed-adverse-effect levels to no observed-adverse-effect levels, the use of such factors does not characterize uncertainty. There is a lack of transparency as to the basis of those factors and whether they mix policy-based assumptions with science-based assessments. Furthermore, a user of the resulting dose-response estimates has no information regarding the quantitative range of uncertainty.

Others have illustrated methods that could be used to quantify uncertainty in dose-response assessment, but such techniques are not reviewed, considered, or applied in EPA's draft assessment of tetrachloroethylene. We mention a few illustrative examples of techniques that others have explored. Evans et al. (1994) demonstrated a probability-tree method for quantifying uncertainty associated with low-dose cancer risk. IEc (2006) has demonstrated a method for quantifying uncertainty in concentration-response functions for fine particulate matter that is based on a formal, systematic approach to eliciting subjective probability distributions from multiple carefully selected experts. Small (2008) enumerates an approach that, if implemented, would advance the state of practice in combining multiple sources of uncertainty, including combination based on judgment and data. In this approach, a prior distribution is postulated to the options on a key assumption, such as the one for MOA, or a key choice, such as candidate data sets. Each final risk estimate is a result of a combined set of assumptions and choices propagating through the risk-assessment process tree and is assigned a probability that results from the prior probabilities assigned to each associated assumption and choice. The collection of all final risk estimates will thus cover all admissible combinations of assumptions and choices and will form a probabilistic distribution that quantifies the full range of variation of the risk estimates. Additionally, this probabilistic distribution of risk estimates can be used, with the incorporation of new data, to obtain posterior probabilities for the assumptions and choices involved in each step of risk estimation. With the help of a distribution of risk estimates to reflect the overarching uncertainties and variations, regulatory policy can be less dependent on a principal study or a few data sets. In fact, the risk-management process can use the distributional properties to choose and justify a final risk estimate in the context of this full range of uncertainties and variations.

Hence, EPA in general and the IRIS program in particular should explore methods for adoption or adaptation to improve the qualitative and quantitative characterization of uncertainty. In general, there should be both well-structured

qualitative assessment of uncertainties and quantitative assessments wherever possible. Preference should be given to quantitative assessment as the desirable approach, and justification for the use of qualitative instead of quantitative approaches should be provided. For example, it should be explained why the state of science is adequate to characterize a point estimate but not a range of uncertainty if quantitative methods of uncertainty analysis are not used.

A key way forward in quantifying uncertainty is to accept the role of expert scientific judgment. Such judgment is used routinely to make inferences regarding hazard identification and in developing dose-response characterizations of chemicals. The examples of Evans et al. (1994), IEC (2006), and Small (2008) rely on encoding expert judgment as subjective probability distributions to various degrees. The appropriate selection and application of methods for quantifying uncertainty in dose-response relationships are undergoing development and need additional research from which guidance on best practices can be derived. As an example of the exploratory nature of dealing with uncertainty in dose-response relationships, the 2007 Resources for the Future workshop "Uncertainty Modeling in Dose Response: Dealing with Simple Bioassay Data, and Where Do We Go from Here?" explored a variety of methods for quantifying uncertainty and the needed role of qualitative assessment to deal with aspects of dose-response modeling that are believed not to be amenable to quantification. Some quantitative techniques that were explored were bootstrap simulation and probabilistic inversion with isotonic regression and Bayesian-model averaging to deal with uncertainty in model structure. However, although there is not yet a default method for quantifying uncertainty in dose-response relationships, EPA can and should review and adopt or adapt various methods that are being explored in the scientific community, taking particular note of the possibilities for combining expert judgment and data with Bayesian approaches.

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Appendix A

Biographic Information on the Committee on Tetrachloroethylene

Sam Kacew (*Chair*) is a professor in the Department of Cellular and Molecular Medicine, Faculty of Medicine, and associate director of toxicology at the McLaughlin Centre for Population Health Risk Assessment of the University of Ottawa. His general research interests are in renal, hepatic, and pulmonary toxicology. Recent work has focused on the effects in infants of chemical contaminants in breast milk, the basis of differences between infants and children in responsiveness to chemicals, and the role of confounding factors in toxicity testing. Dr. Kacew is the recipient of several awards for his research and teaching. Most recently, he was awarded the Public Communications Award from the Society of Toxicology for his contribution to broadening public awareness of toxicologic issues through communication in books and public presentations. He is the editor-in chief of the *Journal of Toxicology and Environmental Health*, editor of the *Encyclopedia of Environmental Health*, and North American editor of *Toxicology and Environmental Chemistry*. He has served on numerous scientific expert panels and committees, including service as chair of the National Research Council Committee on Iodotrifluoromethane and member of the Committees on Depleted Uranium, Flame Retardants, and Jet Propulsion Fuel 8. He received his PhD in pharmacology from the University of Ottawa.

Bruce H. Alexander is an associate professor in the Division of Environmental Health Sciences of the University of Minnesota School of Public Health. His research interests are in applied occupational and environmental epidemiology, epidemiologic methods, and global health. Current research includes respiratory health and community exposure to asbestos-contaminated vermiculite; mortality, cancer incidence, and respiratory health in taconite production workers; health effects of occupational exposure to fluorochemicals; health effects of ionizing radiation in the medical field; pesticide exposure assessment in farm families; and the use of biologic markers in epidemiologic research. Dr. Alexander received his MS in environmental health from Colorado State University and his PhD in epidemiology from the University of Washington.

Margit Bleecker is director of the Center for Occupational and Environmental Neurology in Baltimore, Maryland. Her research interests are in clinical industrial neurotoxicology and occupational neurology. She has served on several Institute of Medicine committees, including two terms on the Committee to Review the Health Effects of Vietnam Veterans of Exposure to Herbicides. She received her PhD from the State University of New York Downstate Medical Center and her MD from the University of California, San Francisco School of Medicine. Dr. Bleecker is certified by the American Board of Psychiatry and Neurology.

Gary P. Carlson is professor of toxicology in the School of Health Sciences of Purdue University. His research interests are in examining the relationship between the metabolism of chemicals and their toxic actions, including an interest in activation and detoxification pathways in the liver and other target organs. Current research involves using a variety of techniques, ranging from in vitro assays to animal bioassays, to examine the biochemical mechanisms by which chemical agents exert their toxic and carcinogenic actions. He has served on several National Research Council committees, most recently as chair of the Subcommittee on Toxicologic Assessment of Low-Level Exposures to Chemical Warfare Agents and currently as a member of the Committee on Toxicology and the Committee on Combined Exposures to Hydrogen Cyanide and Hydrogen Monoxide in Army Operations. Dr. Carlson received his PhD in pharmacology from the University of Chicago.

Linda D. Cowan is George Lynn Cross Professor and chair of the Department of Biostatistics and Epidemiology of the University of Oklahoma Health Sciences Center. Her research interests include cardiovascular-disease epidemiology and the relative importance of risk factors in American Indian men and women, neurologic disorders, and perinatal epidemiology. Her recent research includes analysis of risk-factor profiles for early-onset and late-onset coronary heart disease in American Indians, investigation of the role of environmental toxicants and congenital hearing loss, and studies in west Africa of the prevalence of and risk factors for epilepsy associated with neurocysticercosis. Dr. Cowan has served on the National Research Council Committee to Assess the Health Implications of Perchlorate Ingestion and the Institute of Medicine (IOM) Committee to Assess the Safety and Efficacy of the Anthrax Vaccine. She is a member of the IOM Board on the Health of Select Populations. She received her PhD in epidemiology from Johns Hopkins University.

Mary E. Davis is a professor in the Department of Physiology and Pharmacology of the West Virginia University Health Sciences Center. Her research interests are in the toxicology of environmental and occupational pollutants, including water-disinfection byproducts, halogenated solvents, and arsenic. She is particularly interested in mechanisms of toxicity in the liver, kidneys, and vascular system. Dr. Davis was treasurer of the Society of Toxicology and is a former

president of the society's Allegheny-Erie Regional Chapter. She has served on the U.S. Environmental Protection Agency Science Advisory Board and the editorial boards of *Toxicology* and *Toxicology and Applied Pharmacology*. She was a member of the National Research Council Committee on Assessing Human Health Risks of Trichloroethylene. She received her PhD in pharmacology from Michigan State University.

H. Christopher Frey is a professor in the Department of Civil, Construction, and Environmental Engineering of North Carolina State University. His research interests are in energy and environmental systems, specifically the development and application of methods for quantifying variability and uncertainty and for sensitivity analysis in system models. He has also been involved in exposure and risk analysis, particularly with regard to criteria pollutants, hazardous air pollutants, and particulate matter. Dr. Frey is a former president of the Society for Risk Analysis. He received his MS in mechanical engineering and his PhD in engineering and public policy from Carnegie Mellon University.

Joseph R. Landolph, Jr. is an associate professor of molecular microbiology and immunology and pathology at the Keck School of Medicine of the University of Southern California (USC). He also holds an appointment as associate professor of molecular pharmacology and pharmaceutical sciences in the USC School of Pharmacy. His research interests are in the molecular biology of chemical carcinogenesis induced by nickel and chromium compounds, specifically the processes of oncogene activation and tumor-suppressor gene inactivation in chemically induced neoplastic cell transformation. Other chemicals studied include carcinogenic arsenic compounds and polycyclic aromatic hydrocarbons. Dr. Landolph has held a number of leadership positions in the Society of Toxicology; he has been vice-president, president, and councillor of the Metals Specialty Section and councillor of the Carcinogenesis Specialty Section. He has previously served as a member of the U.S. Environmental Protection Agency (EPA) Human Health Strategies Review Committee and is a member of the Science and Technology Achievement Awards Committee and of the Drinking Water Committee of the EPA's Scientific Advisory Panel. He has served as a member of the Human Health Strategies Review Subcommittee of EPA's Board of Scientific Counselors. He received his PhD in chemistry from the University of California, Berkeley.

David C. McMillan is an associate professor in the Department of Cell and Molecular Pharmacology of the Medical University of South Carolina. His research interests are in the toxicity of drugs and environmental chemicals in erythrocytes and the liver. Current research is directed toward understanding the mechanisms underlying hemolytic anemia induced by drugs. Another line of research is aimed at understanding the role of metabolism in the carcinogenicity of trichloroethylene, specifically how known genetic variation in enzymes responsible for trichloroethylene metabolism alters the rates of production and the

amounts of carcinogenic metabolites produced after exposure. Dr. McMillan received his PhD in pharmacology and toxicology from the University of Arkansas for Medical Sciences, and he is a diplomate of the American Board of Toxicology.

M.E. (Bette) Meek is the associate director of chemical risk assessment at the McLaughlin Centre of the University of Ottawa on interchange from Health Canada, where she managed the Existing Substances Division of the Safe Environments Programme of Health Canada. Her research interests are in hazard and risk assessment of chemical contaminants in the general environment. She led the development of approaches to establishing priorities for health assessment among the 23, 000 substances on the Canadian Domestic Substances List and approaches to in-depth risk assessment of high-priority substances. That included the introduction of novel predictive methods for exposure and hazard characterization, multimedia exposure estimation, chemical-specific adjustment factors for nonneoplastic effects, measures of potency for carcinogens, and robust models of peer engagement. More recently, she has been involved in the development of weight-of-evidence frameworks for mode of action based on consideration of mechanistic data in risk assessment. Dr. Meek has served as an adviser in those and related subjects to international scientific organizations (including the World Health Organization, the Organisation for Economic Cooperation and Development, the International Life Sciences Institute, and the International Labour Organization). She received her MSc in toxicology from the University of Surrey in the United Kingdom and her PhD in risk-assessment sciences at the University of Utrecht in the Netherlands.

M. Christopher Newland is Alumni Professor in the Department of Psychology of Auburn University. His research interests include the neurobehavioral toxicity of heavy metals, specifically the neurotoxicity of methylmercury during early development and aging, and behavioral pharmacology. He has served on advisory panels for the U.S. Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease Registry, and the National Research Council, where he has participated in reviews of the Neurotoxicology Division of the EPA Health Effects Laboratories and the neurotoxicity of elemental mercury, methylmercury, and manganese. His research has been supported by the National Institute of Environmental Health Sciences, National Institute on Drug Abuse, and EPA. He was a member of the Neurotoxicology and Alcohol Scientific Review Group. Dr. Newland is past president of the Neurotoxicology Specialty Section of the Society of Toxicology and past president of the Behavioral Toxicology Society. He has served on several editorial boards and is associate editor of *Neurotoxicology*. He received his MS and PhD in experimental psychology from the Georgia Institute of Technology and had postdoctoral fellowships in environmental health sciences (now environmental medicine) at the University of Rochester.

Julia B. Quint a research scientist, retired as chief of the Hazard Evaluation System and Information Service, Occupational Health Branch, of the California Department of Public Health. She was involved in identifying and evaluating reproductive toxicants, carcinogens, and other workplace chemical hazards and in developing strategies to protect workers, communities, and the environment from the hazards of toxic chemicals. Dr. Quint is a member of the California Environmental Contaminant Biomonitoring Program Scientific Guidance Panel and on the California Division of Occupational Safety and Health's Health Expert Advisory Committee for the Development of Permissible Exposure Limits for Airborne Contaminants in the Workplace. She received her PhD in biochemistry from the University of Southern California.

Gary L. Rosner is a professor of biostatistics at the University of Texas MD Anderson Cancer Center. He also holds an adjunct professorship in the Department of Statistics at Rice University and is a member of the faculty of the University of Texas at Houston Graduate School of Biomedical Sciences. His research interests are in population pharmacokinetics, pharmacodynamic modeling, pharmacogenetics, clinical-trial design, and Bayesian methods. Dr. Rosner has developed methods for analyzing complex biomedical data, such as those arising from population-based studies of the pharmacokinetics and pharmacodynamics of anti-cancer agents. He received his master's in applied mathematical sciences in applied mathematical sciences from Rice University and his ScD in biostatistics from Harvard University.

Ivan Rusyn is an associate professor in the Department of Environmental Sciences and Engineering of the University of North Carolina at Chapel Hill and associate director of the curriculum in toxicology. His research involves applying molecular, biochemical, genetic, genomic, and computational approaches to the understanding of the mechanisms of environmental-agent-related organ injury and carcinogenesis. Recent work has focused on the molecular mechanisms of phthalate-induced carcinogenesis, mechanisms of ethanol-induced hepatic toxicity based on the latest knowledge of the genetic diversity of the mouse as a model organism, and genomic and genetic analysis of hepatic and renal toxicity of trichloroethylene to determine what genetic variants correlate with susceptibility or resistance to hepatic disease. Dr. Rusyn received his MD from the Ukrainian State Medical University in Kiev and his PhD in toxicology from the University of North Carolina at Chapel Hill.

Rolf Schulte-Hermann is emeritus professor of toxicology at the Medical University of Vienna. He was head of the Research Unit Chemical Safety and Cancer Prevention, and, from 1985 to 2004, director of the Institute of Cancer Research at the University of Vienna. His research interests are focused on regulation of organ growth, tumor initiation and promotion, non-genotoxic carcinogens, and role of the microenvironment in chemical carcinogenesis. Major scientific achievements include the discovery of apoptotic cell death during or-

gan regression and carcinogenesis and of apoptosis inhibition by tumor promoters. In 1991 he founded the Austrian Society of Toxicology and served as chairman until 2009. He is director of the Postgraduate Course in Toxicology/Chemical Safety in Vienna since 1993. He served as member of numerous national and international advisory committees. Dr. Schulte-Hermann received his PhD in pharmacy from the Free University Berlin and his MD from the University of Marburg, Germany.

Irvin R. Schultz is a toxicologist in the Marine Sciences Laboratory of Pacific Northwest National Laboratory, operated by Battelle for the U.S. Department of Energy in Sequim, Washington. He also holds an appointment as an adjunct assistant professor in the Department of Biology of the University of Idaho. His research interests cover both ecologic and human health issues. Highlights of his research efforts include studies of the disposition of drinking-water disinfection byproducts in human volunteers, nonhuman primates, and rodent models; development of physiologically based toxicokinetic models to describe the chemical dosimetry and estrogenic activity of xenobiotics; the metabolism and disposition of environmental pollutants in fish, with an emphasis on allometric and interspecies scaling; and the disposition and bioavailability of inorganic and organometallic compounds in fish. Dr. Schultz received his PhD in pharmacology-toxicology from Washington State University.

Robert Snyder is associate dean for research of the Ernest Mario School of Pharmacy of Rutgers University and was a professor and chair of the Department of Pharmacology and Toxicology of Rutgers College of Pharmacy, director of the Environmental and Occupational Health Sciences Institute, director of its Division of Toxicology, and director of the Graduate Program in Toxicology. His research interests are in solvent toxicology, chemically induced bone marrow depression, hepatic toxicity, chemical carcinogenesis, and drug metabolism. He has done extensive work on benzene leukemogenesis. Dr. Snyder is a former president of the American College of Toxicology and has served on several committees of the National Research Council, most recently on the Committee for Review and Assessment of the Army Non-Stockpile Demilitarization Program: Workplace Monitoring.

Roberta F. White is a professor and chair of the Department of Environmental Health of the Boston University School of Public Health. She also is associate dean of research and holds appointments in the Department of Neurology and the Department of Psychology of the university. Her research interests are in the effects of exposures to industrial chemicals and chemical pollutants on brain function on the basis of behavioral measures and neuroimaging techniques. She has studied behavioral and imaging correlates of occupational lead exposure and environmental exposure to methylmercury, structure-function relationships revealed by visuospatial tests, solvent exposures of children and adults, and effects

of prenatal pesticide exposure in farm workers in South Africa. Dr. White received her PhD in clinical psychology from Wayne State University.

Luoping Zhang is an associate adjunct professor in the Division of Environmental Health Sciences of the University of California, Berkeley. Her research interests are in mechanisms of bone marrow toxicity caused by benzene and other toxic chemicals, application of fluorescent in situ hybridization as a biomarker in studies of childhood leukemia and other types of cancer, and application of gene-expression profiling in molecular epidemiology. She received her MS in biochemistry from Huazhong University of Science and Technology in the People's Republic of China and her PhD in biochemical toxicology from Simon Fraser University, in British Columbia, Canada.

Yiliang Zhu is a professor in the Department of Epidemiology and Biostatistics of the University of South Florida College of Public Health and director of the college's Center for Collaborative Research. His current research is focused on quantitative methods in health risk assessment, including physiologically based pharmacokinetic models, dose-response modeling, benchmark-dose methods, and uncertainty quantification. He also conducts research in disease surveillance, health-outcome evaluation, and health-care access and use in developing countries. Dr. Zhu was a member of the National Research Council Committee on EPA's Exposure and Human Health Assessment of Dioxin and Related Compounds. He received his MS in statistics from Queen's University and his PhD in statistics from the University of Toronto.

Appendix B

Dissenting Statement and Rebuttal

Dissenting Statement on Mode of Action of Tetrachloroethylene in Mouse Hepatocarcinogenesis

By Rolf Schulte-Hermann

The authors of the Integrated Risk Information System (IRIS) draft conclude in Chapter 4.4.

- That peroxisome proliferator-activated receptor-alpha (PPAR α) activation is not the primary mode of action (MOA) for tetrachloroethylene-induced hepatocarcinogenesis in mice.
- That the specific mechanisms or MOAs for hepatocarcinogenesis are not known.
- That it is highly likely that more than one mechanism is operative.

That conclusion is supported in Chapter 6 of the present committee review of the IRIS draft although some deficiencies in the draft are mentioned. They include the lack of coherent flow and an imbalance in critiquing the view that the PPAR α MOA is not relevant for human carcinogenesis. This committee member concurs with the criticisms.

However, the member disagrees with the conclusions quoted above. In the members' opinion, the weight of evidence strongly favors a key role of PPAR α activation in tetrachloroethylene-induced hepatocarcinogenesis in mice; furthermore, this MOA lacks relevance for human hepatocarcinogenesis. Because of the deficits in the respective presentation in the IRIS draft, the following paragraphs will briefly compile the essential data supporting the PPAR α MOA

for tetrachloroethylene, the role of trichloroacetic acid (TCA) as the major responsible metabolite of tetrachloroethylene, the potential roles of other MOAs, new mechanistic data supporting the lack of relevance of the PPAR α MOA for humans. The author hopes that the arguments collected in this dissent will be helpful in revising the IRIS draft.

EVIDENCE THAT TETRACHLOROETHYLENE AND TRICHLOROACETIC ACID ARE PEROXISOME PROLIFERATORS

Relevance of Trichloroacetic Acid vs Dichloroacetic Acid

Both TCA and dichloroacetic acid (DCA) are peroxisome proliferators. TCA is the major metabolite found in the body after exposure to tetrachloroethylene. It is eliminated slowly and therefore accumulates to some extent. In contrast, DCA is present in only tiny amounts after tetrachloroethylene exposure because of low formation and more rapid elimination (IRIS draft, Chapter 3). Thus, after tetrachloroethylene administration in mice, DCA concentrations in blood were below 10 or 25 $\mu\text{g/mL}$ in the initial hours and then undetectable and were undetectable in the liver in the presence of high TCA concentrations (up to 150 $\mu\text{g/mL}$ or 150 $\mu\text{g/g}$) (Philip et al. 2007; see below for experimental details). TCA and DCA have similar potency as hepatic carcinogens and tumor promoters (Bull 2000; Bull et al. 2004). Overall, therefore, DCA probably contributes little to PPAR α -mediated effects of tetrachloroethylene. Other metabolites of tetrachloroethylene are not known to be peroxisome proliferators. The arguments related to the PPAR α MOA should therefore focus on TCA.

Peroxisome Proliferator-Activated Receptor-Alpha Transactivation

Tetrachloroethylene (up to 5 mM) did not transactivate mouse and human PPAR α in cells transfected with the PPAR genes. Likewise, chloral hydrate and trichloroethanol, minor metabolites of tetrachloroethylene, did not activate PPAR α . In contrast, TCA was active at 1 and 5 mM but not at 0.1 mM. Activity was considerable at 1 mM, suggesting that the lowest observed-adverse-effect level (LOAEL) for binding activity is distinctly below 1 mM (Zhou and Waxman 1998; Maloney and Waxman 1999). The maximal activation was only about 50% of that of Wy 14643, a strong activator, but similar to that of mono-(2-ethylhexyl) phthalate, the carcinogenic metabolite of di(2-ethylhexyl) phthalate (DEHP). Mouse PPAR γ displayed little, and human PPAR γ no, responsiveness to TCA. DCA transactivated PPAR α with somewhat less potency than TCA, but it showed no effect on mouse or human PPAR γ (Zhou and Waxman 1998; Maloney and Waxman 1999). In another study (Walgren et al. 2000), TCA but not DCA was found to activate mouse PPAR α at 4 mM.

Tetrachloroethylene as a Peroxisome Proliferator

Tetrachloroethylene induces in mouse liver responses that are known to be mediated by PPAR α —such as a 4-fold increase in CN-insensitive palmitoyl-CoA oxidation (PCO), morphologic evidence of peroxisome proliferation based on morphometric analysis, and hepatomegaly—at doses of 1,000 mg/kg by gavage for 10 days or 200 and 400 ppm by inhalation 6 hours/day for 14, 21, or 28 days (Goldsworthy and Popp 1987; Odum et al. 1988). Those effects also occurred, although much more weakly, in rats (Goldsworthy and Popp 1987; Odum et al. 1988). Dose-dependent increases in hepatomegaly and (not significantly) hepatocyte proliferation after oral treatment of mice were reported by Schumann et al. (1980) and Buben and O'Flaherty (1985). In male mice, tetrachloroethylene at daily oral doses of 150, 500, and 1,000 mg/kg transiently and dose-dependently increased hepatocyte DNA synthesis at 7 and 14 days; at 30 days, the increase was nearly gone (Philip et al. 2007). Tetrachloroethylene itself does not bind to PPAR α (see above), so PPAR α -mediated responses should be due to active metabolites, predominantly TCA. In the study by Odum (1988), 200 ppm, the higher dose in the NTP (1986) carcinogenicity study, induced pronounced increases in PCO and peroxisome proliferation that suggested that the NOAEL was much lower. Obviously, doses of tetrachloroethylene that are in the range of the carcinogenic doses are transformed to metabolites (mainly TCA) in amounts sufficient to activate PPAR α in mouse liver. Evidence supporting the role of TCA is presented later.

Trichloroacetic Acid as a Peroxisome Proliferator and Hepatocarcinogen in Mice

TCA was shown in numerous studies to induce PPAR α -mediated responses, such as PCO increases, in the livers of mice of both sexes and to produce liver tumors in mice (Goldsworthy and Popp 1987; Pereira 1996; Bull 2000; Bull et al. 2002; DeAngelo et al. 2008; further references in the IRIS draft). In the first days of administration, TCA induced liver enlargement and an increase in hepatocyte DNA synthesis in male and female mice (Dees and Travis 1994; Pereira 1996; Stauber and Bull 1997). Effects were present when TCA was given at 100 mg/kg orally over 11 days and showed some increase with dose up to 1,000 mg/kg (Dees and Travis 1994). With continued treatment, the enhancement of DNA synthesis disappeared and was reversed to depression (Pereira 1996; Stauber and Bull 1997). TCA induction of the peroxisomal enzymes PCO and acyl-CoA oxidase (by RNA expression) and of CYP 4a depended on the presence of the PPAR α gene and were not seen in PPAR α -null mice (Laughter et al. 2004). Some studies reported increased lipid peroxidation by TCA (Bull et al. 1990; Larson and Bull 1992; Austin et al. 1996). An increase in 8-OHdG was not found after TCA (Parrish et al. 1996) or after tetrachloroethylene (Toraason et al. 1999).

Hepatic tumorigenesis after TCA administration was studied mostly in male mice but was also demonstrated in female mice (Pereira 1996). TCA was found to promote hepatic-tumor development efficiently in mice after initiation by 1-methyl-1-nitrosourea or vinyl carbamate (Pereira and Phelps 1996; Bull et al. 2004). Foci of altered cells (presumably preneoplastic lesions) and tumors were predominantly basophilic and did not express glutathione *S*-transferase-pi (GSTP), as found with other peroxisome proliferators (Pereira 1996; Pereira and Phelps 1996; Stauber and Bull 1997). Clonal expansion of anchorage-independent hepatocytes obtained from male B6C3F1 mice by administration of TCA in vitro was also reported (Stauber et al. 1998).

In a recent lifetime dose-response study, DeAngelo et al. (2008) found that the TCA-induced increase in PCO correlated with tumor induction, and a linear association occurred between the two effects. A TCA NOAEL of 6 mg/kg per day and a LOAEL of 58-68 mg/kg were reported.

Evaluation of Effects of Trichloroacetic Acid (TCA) and Tetrachloroethylene for Consistency with Key Events

Klaunig et al. (2003) have defined seven key events in the PPAR α MOA of rodent hepatocarcinogenesis. TCA was found to induce most of the key events in mice:

1. **Causal relationship** to tumor formation:
 - a. Direct activation of PPAR α (resistance to induction of key events in PPAR α -null mice).
 - b. Transient increase in hepatocyte DNA synthesis.
 - c. Selective clonal expansion of the putative preneoplastic lesions and of tumors.
2. **Associative relationship** to tumor formation:
 - a. Peroxisome proliferation as indicated by morphologic and biochemical studies (high weight of evidence and specificity for association with tumorigenesis [Klaunig et al. 2003]).
 - b. Hepatocyte oxidative stress (lipid peroxidation) (low weight of evidence and specificity for association [Klaunig et al. 2003]).
 - c. Inhibition of gap junctional intercellular communication (GJIC) by TCA in a model with lucifer yellow. The same result was obtained with tetrachloroethylene (Benane et al. 1996).
 - d. Dependence on Kupffer cells has apparently not been studied directly after TCA administration. However, that is not a serious deficiency for the purpose of this discussion, because the specificity of Kupffer-cell dependence is low (Klaunig et al. 2003).

This set of results was generated in several studies, and dose-response and temporal relationships are consistent with the observation of tumors. In the absence

of evidence on genotoxicity and other plausible MOAs, the induction of 6 of the 7 key events provide strong evidence of a PPAR α -dependent MOA of TCA-induced mouse hepatocarcinogenesis. The same conclusion was reached by the National Research Council's Committee on Human Health Risks of Trichloroethylene (2006).

Data on tetrachloroethylene are less comprehensive. An NOAEL and a LOAEL and studies in PPAR α -null mice are not available. Nevertheless, the PPAR α MOA is considered probable on the basis of the induction of several key events in mouse liver, including transient increases in DNA synthesis, lipid peroxidation, inhibition of GJIC, and, most important, peroxisome proliferation, an event highly specific for PPAR α activation. A major argument supporting the PPAR α MOA of tetrachloroethylene is related to the role of TCA as the active metabolite, as will be shown below according to several lines of evidence.

SPECIES DIFFERENCES SUPPORTING THE ROLE OF TRICHLOROACETIC ACID AS THE ACTIVE METABOLITE OF TETRACHLOROETHYLENE

Rats are less sensitive than mice to peroxisome-proliferator effects of the same doses of tetrachloroethylene (Goldsworthy and Popp 1987; Odum et al. 1988) and do not develop hepatic tumors in response to it (NCI 1977; NTP 1986; JISA 1993) or to TCA at doses up to 364 mg/kg per day for 104 weeks (DeAngelo et al. 1989, 1997). Those differences can be explained by the kinetics of tetrachloroethylene in the two species. Mice metabolize the agent and form TCA at concentrations several times higher than do rats (Schumann et al. 1980; Reitz et al. 1996). Thus, the area under the curve (AUC) for blood TCA after exposure to tetrachloroethylene at 400 ppm for 6 hours was 6.7 times higher in mice than in rats (Odum et al. 1988). In addition, mice are more sensitive than rats to induction of peroxisome proliferation by TCA. That may, at least partially, be due to the 10-fold higher binding capacity of rats' than of mice's plasma proteins for TCA (maximal binding capacity, 283 μ M in rats and 29 μ M in mice). As a result, the proportion of TCA available for uptake by the liver will be less in rats than in mice and will produce a weaker response in rats (Lumpkin et al. 2003). The weak peroxisome-proliferator effect seen in rats is obviously insufficient for hepatic-tumor formation. Numerous examples show that low levels of induction of peroxisomes are not necessarily associated with hepatic tumorigenesis (Klaunig et al. 2003). Overall, the striking differences between responses of mice and of rats to tetrachloroethylene can be explained by assuming TCA as the active principle.

CARCINOGENICITY STUDIES WITH TETRACHLOROETHYLENE AND TRICHLOROACETIC ACID

Hepatocarcinogenic doses of tetrachloroethylene in mice are displayed in

Tables 1 and 2. Doses that do not increase rates of hepatocarcinoma were not tested in National Cancer Institute (NCI) and National Toxicology Program (NTP) studies. Rats treated in parallel bioassays did not develop hepatic tumors.

Long-term exposure to TCA was shown to result in hepatic-tumor formation in mice (Table 3A) but not rats (DeAngelo et al. 1997). DeAngelo et al. (2008) exposed male B6C3F1 mice to TCA via drinking water at 0.05, 0.5, 4.5, and 5 g/L for 60 and 104 weeks (Table 3B). Daily doses calculated were 6-8, 58-68, and 572-602 mg/kg. The work consisted of three parts conducted in two Environmental Protection Agency (EPA) laboratories. The authors reported significant increases in the prevalence and multiplicity of hepatic tumors in the two higher dose groups. A TCA NOAEL of 6 mg/kg per day and a LOAEL of 58-68 mg/kg per day were derived for neoplastic and nonproliferative pathology.

Somewhat surprisingly, the IRIS draft does not mention parts 1 and 2 of the study by DeAngelo et al. (2008), which is therefore presented completely in Table 3B. The selection of only one of the two 104-week bioassays has important implications for modeling in Appendix 4A of the IRIS draft because the control group selected shows a dramatically higher hepatic-tumor incidence (64% vs 12%; see parts 3 and 2 in Table 3B). Use of the low-tumor control would increase the fraction of animals affected by TCA (Figure 4A-1 of the IRIS draft) and increase the calculated carcinogenic potency of TCA. To add to the confusion, in the publication of DeAngelo et al. (2008), the allocation of controls and treated groups in parts 2 and 3 of the study is at variance between the methods section and Table 6 of the results section. That discrepancy should be resolved, and all pertinent data should be used in revising the IRIS document. At present, the validity of the modeled TCA potency data as used in Appendix 4A is questionable.

TABLE 1 Carcinogenicity Study in B6C3F1 Mice (Tetrachloroethylene In Corn Oil Was Administered By Gavage 5 Time a Week for 78 Weeks and Followed By an Observation Period of 12 Weeks)

Sex	Bioassay	Dose, mg/kg (TWA)	Carcinoma (Incidence)	Mice at Risk
Male	NCI	0	2	17
		0 (vehicle)	2	20
		536	32	49
		1,072	27	48
Female	NCI	0	2	20
		0 (vehicle)	0	20
		386	19	48
		772	19	48

Source: NCI 1977.

TABLE 2 Incidence of Hepatocellular Adenomas and Carcinomas in B6C3F1 Mice Exposed to Tetrachloroethylene in Two Inhalation Bioassays

Sex	Bioassay	Administered Exposures, ppm	Cumulative Liver Tumors at Week 104		Total at Risk ^a
			Adenomas	Carcinomas	
Male	NTP (1986)	0	12	7	17
		100	8	25	31
		200	19	26	41
	JISA (1993)	0	7	7	13
		10	13	8	21
		50	8	12	19
		250	26	25	40
Female	NTP (1986)	0	3	1	4
		100	6	13	17
		200	2	36	38
	JISA (1993)	0	3	0	3
		10	3	0	3
		50	7	0	7
		250	26	14	33

^aAnimals that died before the first appearance of a hepatocellular tumor, but no later than week 52, were omitted from the totals because they were presumed not to have adequate time in the study to develop tumors.

Source: EPA 2008 (Table 4A-3).

TISSUE CONCENTRATIONS OF TRICHLOROACETIC ACID AFTER ADMINISTRATION OF TETRACHLOROETHYLENE OR TRICHLOROACETIC ACID

A key question in identification of the MOA of tetrachloroethylene-induced hepatic tumors is whether sufficient TCA is formed and available in the target organ for effective induction of peroxisome proliferation and hepatocarcinogenesis. To address that question, a literature search has been conducted for analytic data on TCA concentrations in the liver and, as a surrogate, in the blood. The results are described below and displayed in Tables 4A-D and Figures 1 and 2A-C.

Blood and Liver Concentrations of Trichloroacetic Acid After Administration of Tetrachloroethylene

Blood concentrations of TCA after tetrachloroethylene administration were first analyzed by Odum et al. (1988). After a single exposure at 400 ppm for 6 hours, peak blood concentrations in B6C3F1 mice were 130 µg/mL 3-4 hours after the end of exposure and thereafter declined with a half-life of 7-8 hours. The AUC was calculated (Table 4A).

TABLE 3A Trichloroacetic Acid Drinking-Water Studies in Male Mice: Incidence of Hepatocellular Adenomas and Carcinomas

Source	Weeks of Exposure	TCA Exposure, g/L	Equivalent TCA Exposure, mg/kg-day	N	Incidence of Adenomas	Incidence of Carcinomas	Incidence of Adenomas or Carcinomas	Proportion Responding with Carcinomas
Bull et al. (1990) ^a	37	2	330	11	0	3	3	0.27
	52	0	0	35	0	0	0	0.0
		1	170	11	2	2	NR	0.18
		2	330	24	1	4	NR	0.17
Bull et al. (2002)	52	0	0	20	0	0	0	0.0
		0.5	NR	20	5	3	6	0.15
		2	NR	20	6	3	8	0.15
Herren-Freund et al. (1987)	61	0	0	22	2	0	2	0.0
		5	NR	22	8	7	NR	0.32
Ferreira-Gonzalez et al. (1995)	104	0	0	16 ^b	NR	3 ^b	NR	0.19
		4.5	NR	11	NR	8	NR	0.73
DeAngelo et al. (2008)	104	0	0	56	10	26	31	0.55
		0.05	8	48	10	14	21	0.44
		0.5	68	51	20	32	36	0.71

^aCumulative TCA exposures were provided in grams per kilogram for the mice evaluated at 52 weeks. Those exposures were converted to milligrams per kilogram per day by (1,000 mg/g)/(7 days/[week][52 weeks]).

^bEstimated from the reported proportion responding by selecting the smallest group size and incidence value consistent with the precision of the reported proportion.

NR = not reported.

Source: EPA 2008 (Table 4A-1).

TABLE 3B Complete Presentation of Results of the TCA Carcinogenicity Study of DeAngelo et al. (2008)

Weeks	TCA, g/L	Equivalent TCA, mg/kg	N ^a	Number with Denoma	Number with Carcinoma	Number with Adenoma or Carcinoma
60 (part1)	0 (NaCl)	0	30	7	7	13
	0.05	8	27	15	4	15
	0.5	68	29	21	21	38
	5.0	602	29	38	38	55
104 (part 2)	0 (NaCl)	0	25	0	12	12
	4.5	572	36	59	78	89
104 (part 3)	0 (1.5 g of acetic acid)	0	42	21	55	64
	0.05	6	35	23	40	57
	0.5	81	37	51	78	87

^aNumber of animals examined.

Note: Table 3A from EPA (2008) contains only part 3 and reports higher numbers of animals examined than the publication by De Angelo et al. and somewhat different proportions of carcinomas.

TABLE 4A Blood TCA Concentrations After Tetrachloroethylene Treatment

1) 1x 400 ppm for 6 hours	Odum et al. 1988				
Doses (ppm x 6 hours)	400				
Peak concentrations (µg/mL) ^a	130				
AUC ₀₋₂₄ (µg/mL per hour) ^b	1,760				
2) 1x i.g.	Gearhart et al. 1993				Ratios
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	100	536	1,072	5.36	2.0
Peak concentrations (µg/mL) ^a	23	80	157	3.48	1.96
AUC ₀₋₂₄ (µg/mL per hour) ^b	368	1,317	2,840	3.58	2.16
3) 1x, i.g.; SW mice	Philip et al. 2007				Ratios
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	150	500	1,000	3.33	2.0
Peak concentrations (µg/g) ^a	150	160	170	1.07	1.06
AUC ₀₋₂₄ (µg/mL per hour) ^a	2,583 ^c	2,229	3,208	0.86	1.44
4) 30x, daily i.g.; SW mice	Philip et al. 2007				Ratios
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	150	500	1,000	3.33	2.0
Peak concentrations (µg/g) ^a	75	128 ^c	130	1.71	1.02
AUC ₀₋₂₄ (µg/mL per hour) ^a	864	2197 ^c	2,439	2.54	1.11

^aNumbers read from figure.^bCalculated from figure.^cData of the first two time points were excluded from the calculation.

Note: If not indicated otherwise, male B6C3F1 mice were used. Ratios between doses, peak TCA concentrations, and AUC are indicated. i.g. = intragastric application. Further technical data on the studies is given in the text.

TABLE 4B Blood TCA Concentrations after TCA Treatment

1) 1x i.g., 4-hour fast	Larson and Bull 1992			Ratios	
	1	2	3	1 - 2	
Doses (mg/kg)		20	100	5	
C _{max} (µg/mL)		38 ± 1.65	130 ± 9.9	3.4	
AUC ₀₋₂₄ (µg/mL per hour)		333 ± 9.9	1185 ± 34.7	3.5	
2) 1x i.g., 8-hour fast	Templin et al. 1993			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	5	20	100	4	5
Peak concentrations (µg/mL) ^a	10.1	40.3	80.6	4.0	2.0
AUC ₀₋₂₄ (µg/mL per hour) ^a	87	374	934	4.3	2.5
3a) 1x i.v., 16-hour fast	Gonzalez-Leon 1999				
Doses (mg/kg)			100		
C _{max} (µg/mL)			179 ± 30		
AUC ₀₋₂₄ (µg/mL per hour)			2,516 ± 289		
3b) Pretreatment with TCA at 2 g/L for 14 days, then 1x i.v., 16-hour fast					
Doses (mg/kg)			100		
C _{max} (µg/mL)			214 ± 17		
AUC ₀₋₂₄ (µg/mL per hour)			2,964 ± 418		
4) Drinking water, 14 days	Mahle et al. 2001			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	11.6	110	268	9.5	2.4
Peak concentrations (µg/mL)	10.3	72.9	79.9	7.1	1.1
5) Drinking water for 5 or 14 days	Green 2003 (Data from Sweeney et al. 2009)			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg), 5 days		180	443		2.5
Peak concentrations (µg/mL)		71.6	127		1.8
Doses (mg/kg), 14 days		181	497		2.8
Peak concentrations (µg/mL)		97.5	133		1.4

^aNumbers read from figure.

Note: For explanations, see Table 4A.

TABLE 4C Liver TCA Concentrations after Treatment with Tetrachloroethylene

1) 1x, i.g.; SW mice	Philip et al. 2007			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	150	500	1,000	3.33	2.0
Peak concentrations (µg/g) ^a	53	100	175	1.89	1.75
AUC ₀₋₂₄ (µg/mL per hour) ^a	956	1,690	3,233	1.77	1.91
1) 30x, daily i.g.; SW mice	Philip et al. 2007			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	150	500	1,000	3.33	2.0
Peak concentrations (µg/g) ^a	25	34	42	1.36	1.24
AUC ₀₋₂₄ (µg/mL per hour) ^a	388	563	694	1.45	1.23

^aNumbers read from figure.

Note: For explanations, see Table 4A.

TABLE 4D Liver TCA Concentrations after Treatment with TCA

2) 1x i.g., 8-hour fast	Templin et al. 1993			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	5	20	100	4	5
Peak concentrations (µg/g)	6.4	21.1	28.4	3.3	1.3
AUC ₀₋₂₄ (µg/mL per hour)	55	199	386	3.6	1.9
4) Drinking water, 14 days	Mahle et al. 2001			Ratios	
	1	2	3	1 - 2	2 - 3
Doses (mg/kg)	11.6	110	268	9.5	2.4
Peak concentrations (µg/mL)	6.2	48.2	61.6	7.77	1.28

Note: For explanations, see Table 4A.

Gearhart et al. (1993) administered a single dose of tetrachloroethylene to male B6C3F1 mice by gavage in corn oil at of 0.1, 0.536, and 1.072 mg/kg. The two higher doses correspond to those used in the NCI oral-carcinogenicity study (Table 1). TCA reached peak blood concentrations of 23, 80, and 157 mg/l; these and the AUC are shown in Table 4A.

In a similar study of male Swiss Webster mice, Philip et al. (2007) applied tetrachloroethylene in aqueous gavage (with Emulphor) daily in three dosages (150, 500, and 1,000 mg/kg) for up to 30 days. Concentrations of tetrachloroethylene, TCA, DCA, and trichloroethanol were analyzed after one and 30 treatments. After the first treatment, peak blood TCA was similar with all three dosages. After 30 doses of tetrachloroethylene at 150 mg/kg, blood TCA ranged from 35 to 75 µg/mL in the 24-hour period, and after 500 and 1,000 mg/kg, from 50 to 135 µg/mL. Peak concentrations and the AUC are displayed in Table 4A. Peak hepatic TCA and AUC tended to be lower than the corresponding blood concentrations, particularly after 30 days of treatment (Table 4C).

Table 4 also shows ratios between different doses compared with ratios between the corresponding peak concentrations and AUC values. Although tissue concentrations in general increased with dose, the relative difference tended to decrease with increasing dose. That reflects the well-known fact that the metabolism of tetrachloroethylene is saturable (Buben and O'Flaherty 1985; Reitz et al. 1996).

Blood and Liver Trichloroacetic Acid Concentrations After Administration of Trichloroacetic Acid

TCA concentrations after administration of TCA in mice and rats have been measured in several studies. After a single oral dose of 20 or 100 mg/kg ¹⁴C-TCA in male B6C3F1 mice, TCA C_{max} in blood were 38 and 130 µg/mL. Half-lives (T_{1/2}) were 4.2 and 5.8 hours; for AUC data, see Table 4B (Larson and Bull 1992). In male B6C3F1 mice treated orally with TCA at single doses of 5, 20, and 100 mg/kg, peak blood concentrations were 10.1, 40.3, and 80.6 µg/mL, respectively, and the half-life was 5.4-6.4 hours. Liver concentrations—6.4, 21.1, and 28.4 µg/g—were lower than blood concentrations; it was suggested that this result from plasma-protein binding of TCA. For AUC data, see Tables 4B and D. Liver:blood AUC ratios decreased with increasing dose (Templin et al. 1993).

When given intravenously to male B6C3F1 mice, a “challenge dose” of TCA at 100 mg/kg resulted in a blood C_{max} of 179 µg/mL and t_{1/2} was 10.0 hours. Other mice received TCA for 14 days at 2 g/L in drinking water. The challenge dose was then administered 16 hours after removal of TCA from drinking water. No significant changes in various kinetic measures occurred: blood C_{max} was 214 µg/mL, t_{1/2} 9.4 hours; metabolism of TCA in vitro was not altered. The authors concluded that pretreatment with TCA does not affect metabolism and pharmacokinetics of TCA (Gonzalez-Leon et al 1999).

In a similar study, male B6C3F1 mice received TCA at 0.08, 0.8, or 2.0 g/L in drinking water; this resulted in daily dose rates of 11.6, 110, and 268 mg/kg. After 14 days, blood TCA was 10.3, 72.9, and 79.9 µg/mL; they were almost identical after 3 days. Liver TCA at 14 days was 6.2, 48.2, and 61.6 µg/mL (Tables 4B and D) (Mahle et al. 2001).

Available studies of organ TCA concentrations used male mice except that Green et al. (cited from Sweeney et al. 2009) found even lower blood concentrations in female B6C3F1 mice exposed to TCA than in male mice.

In conclusion, blood and liver concentrations after TCA treatment in different studies are fairly consistent at similar doses. Liver concentrations were lower than blood concentrations. The data from Larson and Bull (1992), Templin et al. (1993), Mahle et al. (2001), and Green et al. from CTL (cited by Sweeney et al. 2009) concordantly demonstrate that peak blood and liver TCA concentrations and AUC do not increase linearly with dose. Rather, as shown by the ratios in Tables 4B and D, the increments decreased with increasing dose.

Above 100 mg/kg, little further increase in peak blood concentrations is apparent from the experimental data available. That result is graphically presented in Figure 1. Obviously, bioavailability of TCA administered orally does not increase linearly with dose in mice. Incomplete bioavailability of oral TCA is independently supported by the study of Gonzalez-Leon et al. (1999), in which TCA was administered intravenously at 100 mg/kg. Peak concentrations and AUC were about 2.5 times higher than the mean in studies that used oral administration (Table 4B). Obviously, a large portion of a 100-mg/kg dose of TCA administered orally is not systemically available. Elimination kinetics in blood after various doses of TCA were similar and repeated treatment with TCA (for 14 days) did not significantly modify its metabolism and kinetics (Templin et al. 1993; Mahle et al. 2001; Gonzalez-Leon et al. 1999), so a dose-dependent limit on absorption of TCA seems a likely explanation of the reduced bioavailability of oral TCA.

The fraction of TCA bioavailable after oral exposure was modeled by Sweeney et al. (2009) on the basis of blood-concentration data of Mahle et al. (2001) and Green et al. They concluded that the apparent bioavailability of TCA from drinking water is 25% at low doses (12 mg/kg) and declines to less than 10% at high doses (800 mg/kg).

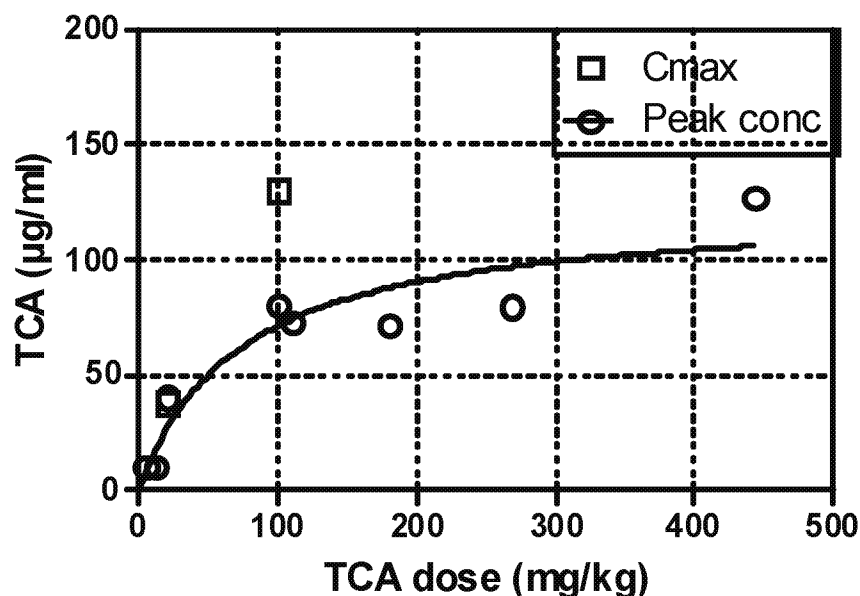


FIGURE 1 Peak TCA concentrations and C_{max} in blood after oral administration of TCA. Source: Data from Table 4B.

Conclusions on the Role of Trichloroacetic Acid in Tetrachloroethylene-Induced Hepatocarcinogenesis

Carcinogenic Potency

The validity of the modeled carcinogenic-potency data on TCA in the IRIS draft (Appendix 4A) is questionable, see earlier section on Carcinogenicity studies with tetrachloroethylene and TCA.

Direct Comparison of Trichloroacetic Acid Concentrations in Blood or Target Organ

Tables 4A and B display the available blood TCA concentrations as determined analytically. Peak TCA concentrations and AUC are similar after application of tetrachloroethylene and TCA at carcinogenic doses or perhaps even higher after tetrachloroethylene than after TCA. That point is illustrated by Figures 2A-C. Likewise, the corresponding liver TCA concentrations are similar after both agents or even higher after tetrachloroethylene. That is convincing evidence that TCA can be formed from tetrachloroethylene and be present in blood and target organ in amounts sufficient to induce peroxisome proliferation and hepatocarcinogenesis.

Modeling the Internal Trichloroacetic Acid Dose

In the IRIS draft, a quantitative comparison between hepatic-carcinoma yields after tetrachloroethylene or TCA treatment and the corresponding internal TCA doses is attempted. Internal TCA after tetrachloroethylene was modeled according to Reitz et al. (1996). For modeling internal TCA after TCA treatment, an absorption rate of 95% was estimated (Section 4A1.2, p.4-205). No reference or reason for that estimate is provided, and no support was found in the literature. Clearly the IRIS estimate is not compatible with the available literature reviewed above, which demonstrates that TCA absorption after oral exposure is incomplete and decreases with increasing dose (Tables 4B and D, Figure 1). Moreover, modeling by Sweeney et al. (2009) suggests that in the 10 mg/kgdose range only 25% of oral TCA becomes bioavailable. Apparently, therefore, the internal TCA doses after TCA treatment that are calculated in the IRIS draft are too high. In consequence, tumor yields predicted to result from TCA formed after tetrachloroethylene (Table 4A-4of the IRIS draft) would be too low. Indeed, when they included their bioavailability data in the model, Sweeney et al. (2009) found that TCA in mice exposed to tetrachloroethylene is sufficient to explain the incidence of hepatic tumors. In conclusion, formation of TCA from tetrachloroethylene is probably sufficient to explain tumorigenesis in mouse liver. That adds substantially to the weight of evidence of a key role of

PPAR α activation in mouse hepatocarcinogenesis by tetrachloroethylene via a metabolism-mediated pathway.

OTHER MODES OF ACTION

The operation of additional, non-PPAR α -mediated mechanisms does not seem necessary to explain hepatocarcinogenesis by tetrachloroethylene but from a scientific point of view cannot be excluded. The question is whether evidence exists which supports a significant contribution of other MOAs to hepatocarcinogenesis.

Cytotoxicity

Tetrachloroethylene causes some hepatotoxicity in mice. It may be due to formation of reactive metabolites, including trichloroacetyl chloride, which have shown protein binding in rodents (Pähler et al. 1999; Green et al. 2001). However, hepatotoxicity has been found to disappear almost completely within 30 days (Philip et al. 2007), and the available long-term carcinogenicity studies revealed little evidence of hepatic damage or inflammation (NTP 1986; JISA 1993). Nevertheless, because the relation between cytotoxicity, inflammation, and cancer is not sufficiently understood, this point should receive attention in future studies. TCA also exerts little hepatotoxicity (Bull et al. 1990; DeAngelo et al. 1989). Overall, current evidence does not indicate that hepatotoxicity of tetrachloroethylene or TCA contributes to hepatocarcinogenesis to a substantial extent. Protein binding in humans was below the level of detection (Pähler et al. 1999).

Genotoxicity

Hypothetically, genotoxic activity could produce initiated hepatocytes, whose development to tumors might be promoted by TCA. Genotoxic activity could thereby enhance the carcinogenic potential of TCA. However, although some metabolites of tetrachloroethylene are genotoxic, there is no convincing evidence of genotoxic or mutagenic effects of tetrachloroethylene in vivo, and no initiating potential has been detected in appropriate assays (committee report, Chapter 5). Thus, a contribution of genotoxicity to hepatic-tumor formation by tetrachloroethylene is not supported by current evidence.

DCA as the Active Metabolite

As described in section on Relevance of TCA vs DCA, substantial contribution to PPAR α -mediated tumor formation is unlikely. The potential MOAs of DCA include genotoxicity, but this activity is weak and probably not relevant at the low levels formed (IARC 2004).

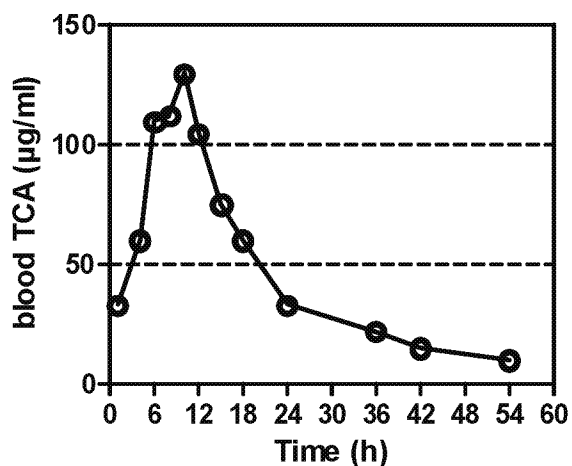


FIGURE 2A TCA concentrations in blood after single exposure of mice to tetrachloroethylene at 400 ppm for 6 hours. Source: Odum et al. 1988. Reprinted with permission; copyright 1988, *Toxicology and Applied Pharmacology*.

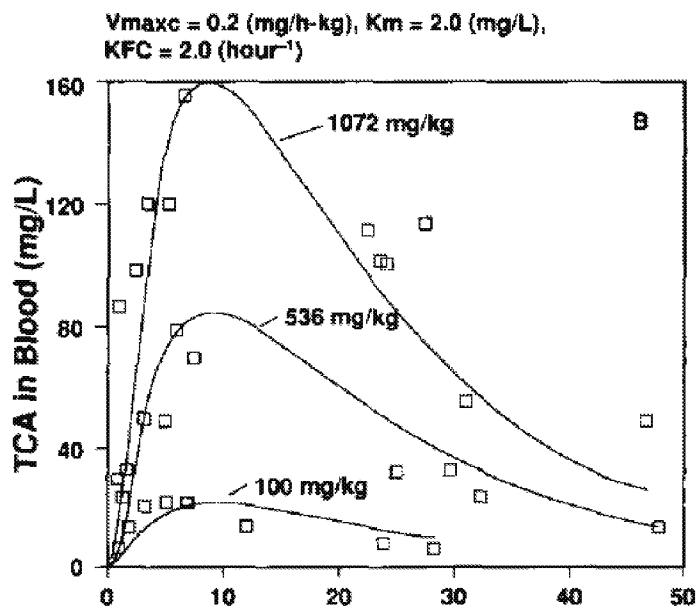


FIGURE 2B TCA concentrations in blood of male mice after single dose of tetrachloroethylene at 0.1, 0.536, and 1.072 mg/kg in corn oil by gavage. Experimental data shown as symbols; computer simulations shown as solid lines. Source: Gearhart et al. 1993. Reprinted with permission; copyright 1993, *Toxicology Letters*.

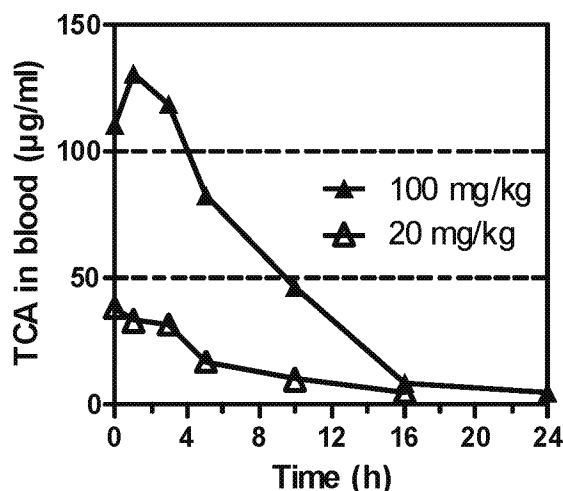


FIGURE 2C TCA concentrations in blood of male mice after single doses of TCA at 20 or 100 mg/kg by gavage. Data read from figure. Source: Larson and Bull 1992. Reprinted with permission; copyright 1992, *Toxicology and Applied Pharmacology*.

Other Mechanisms

Several other effects of tetrachloroethylene or TCA have been discussed as potential MOAs. Among these, changes in DNA methylation occur during PPAR α activation. Therefore, that effect, although not specific for the PPAR α MOA, does not necessarily support a contribution of other MOAs to hepatocarcinogenicity of tetrachloroethylene. TCA slightly transactivated mPPAR γ , but this effect was much weaker than seen with PPAR α and therefore is considered to have little or no relevance in mouse hepatocarcinogenesis. Importantly, TCA had no effect on human PPAR γ (see section on PPAR α Transactivation). In conclusion, there is no evidence available to suggest that MOAs other than PPAR α activation have a significant impact on mouse hepatocarcinoma formation by tetrachloroethylene. Therefore, the weight of evidence supports the PPAR α MOA.

SOME RECENT FINDINGS CONCERNING THE ROLE OF PPAR α ACTIVATION IN MOUSE AND HUMAN HEPATOCARCINOGENESIS

The evidence suggesting that PPAR α activation plays a causal role in rodent hepatic-tumor formation by many peroxisome proliferators but is not relevant for human hepatocarcinogenesis has been compiled in recent reviews (Klaunig et al. 2003; Meek et al. 2003; Peters et al. 2005; EU 2008; Corton 2008).

The authors of the IRIS draft present two recent publications that in their opinion raise questions about the causal relationship between activation of PPAR α and rodent hepatic-tumor formation (p. 4-31). First, Yang et al. (2007) used transgenic mice (LAP-VP16PPAR α) that target constitutively activated PPAR α specifically at hepatocytes. The transgenic mice exhibited various PPAR α -mediated effects—changes in fatty acid metabolism peroxisome proliferation and hepatocyte proliferation—but, surprisingly, not hepatic tumors after 1 year. Transgenic mice showed no hepatocyte hypertrophy and eosinophilia and no induction of proliferation of nonparenchymal liver cells. Those results indicate that PPAR α -dependent induction of hepatocyte proliferation alone is not sufficient for hepatocarcinogenesis and that additional effects, such as activation of nonparenchymal cells, are required. Activation of Kupffer and other nonparenchymal cells had been found necessary for optimal induction of proliferation of normal and preneoplastic hepatocytes (Rose et al. 1997; Parzefall et al. 2001; Hasmall et al. 2001; Drucker et al. 2006). Thus, the study of Yang et al. does not refute the PPAR α MOA but confirms and extends current knowledge.

Second, Ito et al. (2007) found that a low dose of DEHP (0.05% in diet) known to be noncarcinogenic in wild-type mice produced a low rate (26%) of hepatic adenomas in PPAR α -null mice after 22 months. The tumors apparently were induced by oxidative stress and inflammation, as indicated by histopathologic changes and increases in 8-OHdG, NF- κ B, and c-jun RNA, all of which were particularly high in the null mice. Activation of PPAR α can have anti-inflammatory effects, resulting in higher vulnerability to tumorigenesis in PPAR α -null mice (Ito et al. 2007). 8-OHdG was not increased after tetrachloroethylene or TCA (see earlier section on TCA as a Peroxisome Proliferator and Hepatocarcinogen in Mice). Thus, the results of Ito et al. suggest that DEHP, an agent unrelated to tetrachloroethylene, can induce (benign) hepatic tumors through a second, previously unsuspected PPAR α -independent pathway. They do not contradict the causal role of PPAR α activation in many instances of rodent hepatocarcinogenesis induced by peroxisome proliferators, which is supported by overwhelming evidence.

Some important new findings are missing in the IRIS draft. Thus, the generation of transgenic mice in which the mouse PPAR α is replaced by the human counterpart provided substantial progress. The hPPAR α mice were essentially resistant to hepatocarcinogenesis when fed a potent peroxisome proliferator (WY-14643) for 44 weeks, whereas corresponding wild-type mice developed tumors in 38 weeks. Gene-expression analysis for peroxisomal fatty-acid-metabolizing enzymes revealed that both receptors were functional. The findings suggest that structural differences between human and mouse PPAR α are responsible for the different susceptibility of mice and humans to hepatocarcinogenesis by peroxisome proliferators (Morimura et al. 2006).

Furthermore, it was shown that induction of hepatocellular proliferation by peroxisome proliferators involves downregulation of the microRNA let-7c gene by mPPAR α . That in turn allows increased expression of c-myc protein, which is essential for hepatocyte proliferation and tumor formation. Human PPAR α

apparently cannot suppress *let 7c* expression, and *c-myc* was not increased in hPPAR α mice after WY-14643 treatment (Shah et al. 2007; Gonzalez and Shah 2008). Overall, the findings provide mechanism-based support for the concept that the PPAR α MOA of rodent-hepatocarcinoma induction is not relevant to human hepatocarcinogenesis.

SUMMARY

This dissent has critically reviewed evidence related to MOAs of mouse hepatocarcinogenesis after exposure to tetrachloroethylene. The following conclusions can be drawn from findings in the literature:

1. TCA is the major metabolite in the body after exposure to tetrachloroethylene. DCA concentrations in blood and liver were lower than those of TCA by an order of magnitude, or DCA was completely undetectable.

2. TCA transactivates PPAR α , while tetrachloroethylene does not. DCA also activates PPAR α , but, because of its low occurrence, arguments related to the PPAR α MOA should focus on TCA as the dominant active metabolite.

3. Effects of tetrachloroethylene and TCA associated with peroxisome proliferation were compiled and evaluated for consistency with the PPAR α MOA as suggested by Klaunig et al. (2003). TCA induces the three key causal events, as well as peroxisome proliferation, and other associated key events. Data were generated in several studies, and dose-response and temporal relationships are consistent with the observation of tumors. The weight of evidence of this MOA was considered *strong* for TCA (in agreement with the National Research Council trichloroethylene committee) and *probable* for tetrachloroethylene although studies of PPAR α -null mice are not available. Major support of the PPAR α MOA of tetrachloroethylene rests on the role of TCA as the active metabolite.

4. Rats are less sensitive than mice to PPAR α -mediated effects of tetrachloroethylene and do not develop hepatocarcinoma in response to tetrachloroethylene or TCA. That species difference can be explained by kinetic differences in TCA formation and availability in the target organ. In mice, formation of TCA is much higher and binding to plasma proteins much lower than in rats. Therefore, the mouse-rat difference can be explained by assuming that TCA is the active metabolite of tetrachloroethylene.

5. A key question is whether sufficient TCA is produced from tetrachloroethylene to induce peroxisome proliferation and tumor formation in the liver. To address that question, analytic data on blood and liver concentrations of TCA were collected from the literature. The data revealed that peak and AUC levels of TCA in mouse blood after tetrachloroethylene were similar to or even higher than those after TCA when carcinogenic doses of the two agents were compared. That constitutes direct evidence that TCA can be generated from tetra-

chloroethylene and be present in blood and target organ in amounts sufficient to induce peroxisome proliferation and hepatocarcinogenesis.

6. Analytic data from all of five available studies consistently demonstrate that absorption of TCA after oral application is incomplete and decreases with increasing dose. Moreover, published modeling work based on some of those studies suggests that only 25-10% of oral TCA bioavailable. The analytic and modeling data are not compatible with the estimate in the IRIS draft that 95% of oral TCA is absorbed—an estimate apparently not founded on experimental data. Apparently, the internal TCA doses derived from that estimate are too high. Consequently, the tumor yields predicted for tetrachloroethylene-derived TCA would be too low. Indeed, modeling studies taking into account the limited bioavailability of TCA suggest that TCA generated from tetrachloroethylene is sufficient to explain the incidence of hepatic tumors.

In conclusion, the weight of evidence clearly favors a key role of PPAR α activation by TCA in tetrachloroethylene-induced mouse hepatocarcinogenesis.

7. The available evidence does not support a substantial contribution of other MOAs to hepatocarcinogenesis by tetrachloroethylene.

8. Transgenic mice carrying the human PPAR α gene were found to be essentially resistant to hepatocarcinogenesis by a model peroxisome proliferator. This and other recent molecular data provide mechanism-based support for the concept that the PPAR α MOA lacks relevance to human hepatocarcinogenesis.

COMMITTEE REBUTTAL

The committee greatly appreciates the dissenting member's thoughtful and careful review of the scientific literature and presentation of the arguments with respect to the MOA of tetrachloroethylene in mouse hepatic tumors and its relevance to humans. As noted by the dissenter and in Chapter 6 of the committee's report, the committee agrees that the EPA MOA characterization for hepatic cancer is inadequate and should be revised to provide a more focused and integrated analysis of the available evidence on tetrachloroethylene and its metabolites. The dissenter's statement is an attempt to provide an example of how such an analysis might be performed. The committee supports much of the dissenter's approach, but the dissenting member's conclusions go beyond those drawn by the full committee.

The dissenting member holds the opinion that PPAR α mediation of tetrachloroethylene-induced hepatocarcinogenesis in mice is the plausible predominant MOA and that this MOA lacks relevance to human hepatocarcinogenesis. The committee believes that the arguments presented are reasonable and advises EPA to review the considerations presented by the member and the recent literature cited carefully. However, the committee does not support the apparent conclusions regarding mouse hepatic cancer that TCA is the sole carcinogenic metabolite of tetrachloroethylene, that the only MOA of TCA is peroxisome proliferation, and that there is unmistakable concordance in the carcinogenic

potency of tetrachloroethylene in the National Toxicology Program and Japan Industrial Safety Association bioassays and the corresponding studies of TCA. Overall, the committee judges that many gaps in knowledge remain with regard to the MOA of tetrachloroethylene and that the relevance of the peroxisome-proliferator MOA to tetrachloroethylene-induced mouse hepatic cancer and to tetrachloroethylene-induced human hepatic cancer remains hypothetical and requires further rigorous testing.

The committee generally supports the comprehensive literature review and analyses conducted by the dissenting member and recommends that EPA use them when reassessing its own evaluation. However, there are aspects of the dissenter's analysis that the committee believes require more rigorous assessments before definitive conclusions can be drawn. They include the following:

- The committee does not agree that a role of DCA in tetrachloroethylene-induced hepatic carcinogenesis in mice can be ruled out solely on the grounds that it is detected at much lower concentrations than TCA in the blood and liver. First, there are few data on DCA formation from tetrachloroethylene. Second, there is some evidence that DCA is formed via a metabolic pathway that does not involve the liver. Third, there is some debate on whether DCA is formed from TCA. In Chapter 6, the committee stated that the conclusions regarding potential relevance or lack of relevance of DCA to hepatic carcinogenesis by tetrachloroethylene would be strengthened by the comparison of tetrachloroethylene hepatocellular-tumor data with predictions based on DCA carcinogenesis studies (in a way similar to that presented in Appendix 4A of the draft IRIS assessment). Such an analysis would provide a strong quantitative rationale for DCA's potential involvement, or lack thereof, in hepatic cancer.
- A more critical look at the quantitative differences in metabolic activation of tetrachloroethylene to TCA between mouse and rat, species that are generally believed to be almost equally sensitive to peroxisome proliferation, and in induction of hepatic cancer by other compounds in this class should be conducted by EPA. Chapter 6 recommends that EPA consider performing additional analyses with the rat data similar to those done with the mouse in Appendix 4A of the draft and including a table that shows the quantitative differences in affinity to mouse, rat, and human PPAR α of both tetrachloroethylene and its key metabolites in comparison with the known peroxisome proliferators. Such analyses and data would greatly facilitate the discussion of quantitative differences between compounds and species.
- The committee supports the use of the weight-of-evidence analysis and the need for evaluation of the key events in hepatic carcinogenesis by tetrachloroethylene and its key metabolites. However, important knowledge gaps remain to be addressed with regard to key events in the PPAR α MOA, especially those with causal and associative relationship to tumor formation and tetrachloroethylene or its key metabolites (see dissenter's statement and Chapter 6). Indeed, the committee is not yet convinced of the proof of the hypothesis that

the PPAR α MOA is the sole MOA of tetrachloroethylene in inducing mouse hepatic cancer. Hence, it is premature to draw conclusions on the relevance of the PPAR α MOA to tetrachloroethylene-induced human hepatic carcinogenesis.

- The committee agrees that the issues of TCA bioavailability, absorption, and blood and liver concentrations in various exposure scenarios are critical for the consideration of MOA of tetrachloroethylene. The current analysis by EPA is important but is inadequate in its current form. The committee recommends that EPA reconsider the analyses performed and consider using the data suggested by the dissenting member.

- The committee disagrees with the dissenter that the available evidence is sufficient to conclude that other MOAs are unlikely to contribute substantially to hepatocarcinogenesis by tetrachloroethylene. As noted in Chapter 6, the committee recommends that EPA strengthen and clarify the description of the degree, rather than the “significance,” of the contribution of other plausible molecular events, in addition to activation of PPAR α , to mouse hepatic tumors produced by tetrachloroethylene.

- The committee agrees with the dissenter that recent findings reported with PPAR α -null mice (Ito et al. 2007; Takashima et al. 2008; Eveillard et al. 2009), PPAR α humanized transgenic mice (Morimura et al. 2006), and hepatocyte-specific constitutively activated PPAR α transgenic mice (Yang et al. 2007) are valuable contributions to the discussion of the relevance of the PPAR α MOA in human hepatic carcinogenesis. The dissenter cites those studies to draw a conclusion that the PPAR α MOA lacks relevance to human hepatocarcinogenesis. However, alternative conclusions that can be drawn from the studies mentioned above are that the short-term carcinogenesis studies in the PPAR α -null mouse model have important limitations, that activation of PPAR α is necessary but not sufficient for the development of mouse hepatic tumors, and that additional molecular events may be important parts of the peroxisome-proliferator MOA. Thus, the committee believes that it is premature to draw definitive conclusions regarding the relevance of the PPAR α MOA to human hepatocarcinogenesis. In Chapter 6, the committee has encouraged EPA to strengthen the discussion of this matter in the draft IRIS assessment.

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